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Short Communications



THE ROSÉN VON ROSENSTEIN AWARD

In commemoration of the two hundredth anniversary of Nils Rosen von Rosenstein s publication of his famous textbook *Diseases of Children and Their Remedies* a medal was struck in 1964 by the Swedish Paediatric Association It was decided that this medal should be awarded every fifth year to distinguished paediatricians who had worked in the spirit of Rosen von Rosenstein and had contributed significantly to the development of paediatrics

In 1964 the medal was presented to Professors Charles Janeway Boston Guido Fanconi Zurich Arvid Wallgren Stockholm and Arvo Ylppo Helsinki and in 1969 to Professors Josef Houstek Prague Derrick Jelliffe Kingston and Alfred Sundal Bergen

In 1974 the memory of Rosen von Rosenstein was celebrated at a meeting of the Swedish Paediatric Association on 11th May in Uppsala in the Anatomical Theatre of Uppsala s oldest existing university building Gustavianum The medal was presented to Dr Ronald MacKeith London Professor Samuel Fomon Iowa City and Professor Bo Vahlquist Uppsala

REFERENCE

Nils Rosen von Rosenstein and his Textbook on Paediatrics *Acta Paediatr Scand* Suppl *156* 1964

Case Reports

Review Article

Proceedings of Paediatric Societies

Supplements

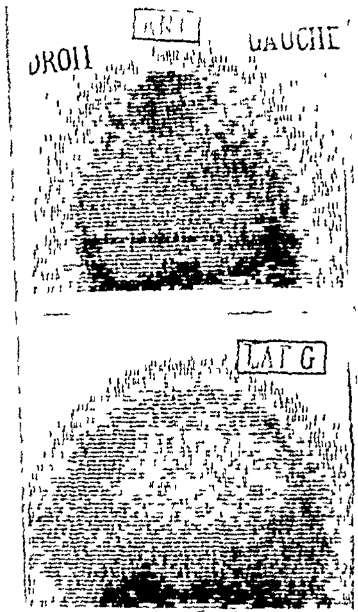


Fig 1 Brain scanning frontal and left lateral views Typical left peripheral hyperactivity in a case of postmeningitic subdural collection In the anterior view there is a widening of peripheral activity In the left lateral view an area of increased activity extends along the periphery from the occipital to the frontal region

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VALUE OF BRAIN SCANNING IN PEDIATRIC SUBDURAL COLLECTIONS

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ABSTRACT Piepsz A Bormans J Segers A Noterman J and Decostre P (Departments of Paediatrics Radioisotopes and Neurosurgery University Hospital Brussels Belgium) Value of brain scanning in pediatric subdural collections Acta Paediatr Scand 64 2 1965 —Eighteen children with subdural collections were submitted to brain scintigraphy By this method idopathic and post traumatic hematomas were detected in 40% of the cases and subdural effusions in 70% of the cases No false negative results were noted in the 3 cases of empyema Several false positive images were recorded most of them following purulent meningitis without any satisfactory explanation Neither the technique of scintigraphy used in the department the dimensions of the skull the age of hematoma nor the presence of membranes seemed to affect the accuracy of the method Compared with the other easily performed examinations (eye fundus EEG Echo) scintigraphy still remains important in the diagnosis of subdural collections in children

KEY WORDS Children brain scintigraphy subdural collections

Subdural collection of fluid is a not uncommon lesion in infancy (12 23) In our Department of Pediatrics an average of 7 children per year undergo each year surgical management for post traumatic or idiopathic subdural collections From January 1971 to June 1972 128 children with purulent meningitis were treated among the 120 who survived subdural complications were noted in 8 3% with the highest incidence for the group under 1 year (30%)

Early diagnosis of subdural collections is essential in children subdural empyema is a fulminating disease rapidly terminating in death if untreated (3) in chronic neglected post traumatic as well as postinfectious collections membranes may be thick and the cerebrum usually shows marked atrophy The removal of subdural membranes provides the maximal insurance against subsequent restriction of cerebral growth development and function (2 17)

Subdural collections have no characteristic clinical picture and their diagnosis is often difficult particularly in older children where subdural taps have become impractical (17)

Since an increasing reliance is being placed on the procedure of scintigraphy the purpose of this study is to determine the reliability of brain scintiscan and to compare it with other diagnostic techniques Brain scintigraphy in subdural collections has been extensively studied in adults but as yet few papers have dealt with this procedure in children Furthermore no distinction has been made between post traumatic hematomas and subdural collections in relation to pyogenic meningitis although their pathogenesis and clinical course are different

In this study the following definitions were used

(*a*) post traumatic or idiopathic subdural hematoma subdural accumulation of sterile

Table 1 *Clinical procedures used in the diagnosis of subdural collections*

Procedures	Abnormal findings	Number of patients tested
Eye fundus	Hemorrhages or papilledema	18
Skull X ray	Fracture or distension of suture	18
EEG	Diminution of amplitude	15
Subdural taps	≥3 ml hyperalbuminous fluid	14
Echoencephalogram	Deviation > 2 mm	9
Cerebral angiography	Peripheral empty space	3
Scan	Peripheral hyperactivity	18

fluid either related to trauma or without any detectable etiology

(*b*) subdural effusion subdural accumulation of sterile fluid in the course of pyogenic meningitis

(c) subdural empyema subdural collection of purulent fluid

MATERIAL AND METHODS

From May 1969 to June 1972 brain scintiscan was performed on 18 children (8 boys and 10 girls) with surgically proven dural collections Amongst those patients 12 were less than 1 year old ? between 1 and ? years ? between 2 and 3 years the remaining ? were adolescents of 13 and 14 years

Preoperative diagnosis was based on clinical history physical examination and procedures as listed in Table 1

Brain scintigraph was performed with a Philips rectilinear scanner equipped with a 5 inch sodium iodide thalium activated crystal lead shielding and appropriate collimation

99 m Tc pertechnetate (22 μCi/kg) was used intravenously after blocking the choroid plexus and salivary glands with oral potassium perchlorate (600 mg) Brain scanning started approximately 10 minutes after radionuclide injection Better immobilization of the head was obtained by using a vacuum hardened cushion One frontal and two lateral views were available *in each* patient The typical scan pattern of dural collection is an abnormal peripheral *increase* in radioisotopic concentration (Fig 1)

The frontal view shows a widening of peripheral activity In the lateral view an area of increased activity extends along the periphery from the occipital to the frontal region Normal scan pattern was considered as false negative when a subdural collection was found at surgery Abnormal scan pattern was considered as false positive when surgery failed to evidence any subdural collection

Flexi-cast d vided shape bag Picker

RESULTS

Twenty nine surgical approaches were performed either by burr holes or by craniotomy Seven had bilateral lesions eleven had unilateral lesions Thus 25 dural collections were discovered 7 postmeningitis effusions 3 empyemas and 15 post traumatic or idiopathic subdural collections To avoid confusion between patient and lesion in the text the word case is related to lesion and not to patient

Membranes were found 18 times they were absent in 3 cases and in 4 cases no data were available

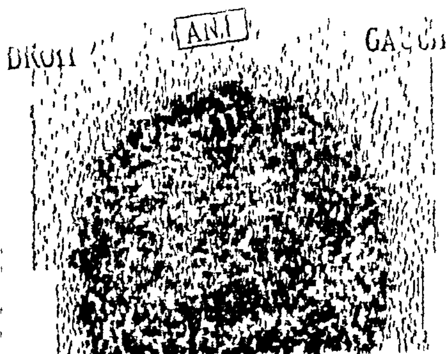
In the 29 operated hemispheres with or without subdural collection 15 scanning patterns were in agreement with the surgical findings (52%) 14 were inconsistent with them In the 25 proven collections 11 were found negative on scintigraph (Table 2)

(a) *Post traumatic and idiopathic collections*

This group concerns 10 children in which 15 dural collections were demonstrated during 17 surgical interventions Preoperative diagnosis was twice proven false in the first case the scan was positive but only a brain

Table 2 *Relationship of brain scanning to surgical demonstration of subdural collections*

	Coherent		Contradictory	
Scanning	Pos or in concl		Pos	Neg
Surgery	Pos	Neg	Neg	Pos
Post traumatic and idiopathic	6	1	1	9
Postmeningitic	5	0	2	2
Empyemas	3	0	0	0
	15		14	



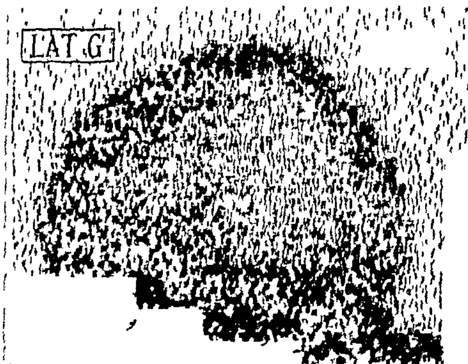


Fig 2 Brain scanning frontal and left lateral views Same aspect as in Fig 1 in a case of pyogenic meningitis with suspicion of subdural complication *No* collection was found at surgery

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[1] Flexi-cast divided shape bag Picker

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	15		14	

Table 3 *Brain scanning in 25 proven subdural collections*

	Results	Number	%
Post traumatic and idiopathic collections	Positive	4	
	Inconclusive	2	40
	Negative	9	60
Postinfectious effusions	Positive	3	
	Inconclusive	2	70
	Negative	2	30
Empyema	Positive	3	100

contusion was discovered in the second case no lesion was found and the scan was negative Scan was considered inconclusive or positive in 6 cases (40%) False negative results were found in 9 cases (60%) (Table 3)

(b) *Postmeningitis effusions*

In 5 children with suspected postinfectious subdural collections surgical exploration of 9 hemispheric subdural spaces showed 7 effusions No lesions were found in 2 cases while scanning pattern was distinctly pathological representing false positive results (Fig 2) In the 7 proven effusions scan was inconclusive or positive in 5 cases (70%) false negative images were found in 2 cases (30%) (Table 3)

(c) *Subdural empyemas*

The scan was positive in the 3 children with proven subdural empyema (Table 3)

DISCUSSION

Radionuclide scanning detects approximately 80% of dural fluid collections in adults (6) Nevertheless wide variations of percentages are observed ranging from 60 to 100% (4 6 16 22) Eleven false positive results were recently published (1) and only 4 of them were explained by malpositioning of the head Scintigraphic experience is very limited in the detection of dural fluid collections in children Most publications report only a few examples (7 11, 15, 18 21) In t group of Kaffer (13) 7 scans out of 9 were positive A more important study has recently been published (5) concerning scintigraphic studies performed on 25 children with subdural collections Scans are positive only in 60% of the cases but no differentiation is reported between dural collections and dural effusions

The present work shows a very similar low percentage of positive results with a global yield of brain scanning reaching 56% but brings out great differences between the 3 precedently defined groups If the rate of accuracy seems to be very similar in empyema (3/3) and in postmeningitis effusions (5/7) only 6 out of the 15 post traumatic or idiopathic subdural collections were detected by brain scanning In this last group however the diagnosis was only once invalidated during surgical approach In this patient the scan showed bilateral hyperactivity while subdural collection was found only on one side and cerebral contusion was detected on the other

During the evolution of purulent meningitis a typical scan pattern of peripherally increased uptake was observed without any surgical complication Except for meningitis itself no other explanation has been found for these false positive scans (recent skull injury skin hematoma) No false negative results were noted in our 3 cases of empyema

In spite of the evident contribution of the scintigraphic technique in the diagnosis of subdural collections one should keep in mind the possibility of false positive and negative records

Table 4 shows the reliability of other techniques in the diagnosis of subdural collections

It must be emphasized (i) that the eye fundus is frequently pathological in post traumatic or idiopathic collections but does not give any positive information when a postmeningitic or purulent collection is sus

Table 4 *Reliability of various procedures in the diagnosis of subdural collections*

	Post traumatic and idiopathic		Postmeningitic		Empyema	
Procedures	Patients	%	Patients	%	Patients	%
Eye fundus	6/10	60	0/5	0	0/3	0
EEG	3/8	37	2/4	50	3/3	100
Echoencephalogram	0/5	0	/3	66	1/1	100
X ray distension of sutures	5/10	50	?/5	40	1/3	33

pected and (ii) the echoencephalogram and the electroencephalogram are often very difficult to interpret Bilateral lesions are constantly missed by these methods

Brain scanning appears as a good tool in the diagnosis of subdural collections It must be combined with other investigations but remains a choice investigation means in the evaluation of such lesions allowing one to restrict the more hazardous cerebral angiography to doubtful cases

In order to explain the inconstancy of scintigraphic results we analysed the importance of the following factors

1 *Scintigraphic technique*

Three hundred scintigraphs were performed on children in our department during the last 3 years The percentage of positivity for overall brain lesions is similar to that found in the literature (11 13 15 18 21 24) and for instance 5 out of 6 posterior fossa tumors were accurately detected (83%) Thus our scintigraphic technique is reliable and does not explain the low scan accuracy in subdural collections

2 *Head circumference*

Distance between midline and periphery of the head is very short in infants and sometimes makes the interpretation of the scintigraphic patterns difficult However no relation was found between the patient s age and the accuracy of the scan

3 *Age of the lesions*

Early lesions in adults do not give positive scans (6 9) The exact duration of the dural collection was carefully evaluated on the basis of the known trauma (7 cases) or of the onset of the meningeal symptoms (8 cases) The interval varied between 1 and 35 weeks and had no influence on the accuracy of the scintigraphy In the present work no relation with the age of hematoma has been found

4 *Presence of membranes*

The exact mechanism of the peripheral crescentic uptake in subdural hematoma has been discussed in several papers and is still a matter of some dispute Some studies (8 14 25) show a diffusion of technetium into the subdural fluid Other studies (6 10 19 20) demonstrate mainly a concentration of isotope into the membrane For these authors accuracy of scan would be in correlation with the well formed subdural membranes In our study no such correlation was found However preliminary experiments suggest a slow diffusion of the pertechnetate ion into the collection and could explain the higher concentration in delayed scan

REFERENCES

1 Arkles L B Andrews J T & Stevens L W A reappraisal of the scan diagnosis of subdural hematomas *Am J Roentgen* *115* 62 1972
2 Benson P M B The prognosis of subdural effu

sions complicating pyogenic meningitis J Pediatr 57 670 1960
3 Bhoudary A M Subdural empyema J Neurosurg 32 35 1970
4 Cine I S Quinn J L & Bucy P C Mercury 197 and Tc 99 m brain scan in the diagnosis of non neoplastic intracranial lesions J Neurosurg 27 119 1967
5 Conway J J & Vollert J M The accuracy of radionuclide imaging in detecting pediatric dural fluid collection Radiology 105 77 1972
6 Cowan R J Maynard C D & Lassiter K R Tc 99 m pertechnetate brain scans in the detection of subdural hematomas J Neurosurg 32 30 1970
7 David R B Beiler D & Hood H Scintillation brain scanning in children Am J Dis Child 112 197 1966
8 Dunbar H S & Ray B S Localisation of brain tumors and other intracranial lesions with radioactive iodinated human albumin Surg Gynecol Obstet 98 433 1964
9 Feindel W Rovit R L & Stephens Newsham L Localisation of intracranial vascular lesions J Neurosurg 18 811 1961
10 Gilson A J & Gargano F P Correlation of brain scans and angiography in intracranial trauma Am J Roentgen 94 819 1965
11 Hirley P J Wagner H N Jr Diagnostic value of brain scanning in children JAMA 221 877 1972
12 Ingraham F O & Matson D D Subdural hematoma in infancy Advances Pediatr 4 231 1949
13 Kaiffer M Naoum A Neimann N Pierson M Robert J La scintigraphie cérébrale au Tc 99 m chez l enfant Arch Fr Pédiatr 28 487 1971
14 Kramer S & Rovit R L The value of Hg 203 brain scans in patients with intracranial hematomas Radiology 83 902 1964
15 Lorentz W B Simon J L & Benua R S Brain scanning in children JAMA 201 5 1967
16 McGinnis K D Eyler W R Dusault L A Kristen K Mercury 203 brain scanning A method of clinical classification Radiology 80 264 1963
17 Matson D D in Neurosurgery of infancy and childhood Ch C Thomas Springfield Illinois 1969 2nd ed
18 Maynard C D & Kelsey W M Brain scanning in the pediatric age group Develop Med Child Neurol 11 69 1969
19 Mealey J Jr Gamma ray image of subdural effusions J Neurosurg 19 934 1962
20 — Radioisotope localisation in subdural hematomas J Neurosurg 20 770 1963
21 — Brain scanning in childhood J Pediatr 69 39 1966
22 Planiol T Radioisotope in neurology World Neurol 2 153 1961
23 Smith M H D Subdural lesions in childhood Advances Pediatr 8 165 1956
24 Tefft M Radioisotopes in malignancies in children JAMA 207 1853 1969
25 Williams C M & Garcia Bengochea F Concentration of radioactive chlormerodrine in the fluid of chronic subdural hematoma Radiology 84 7 1965

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LUNG EXPANSION AND THE FORMATION OF THE ALVEOLAR LINING LAYER IN THE FULLTERM NEWBORN RABBIT

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ABSTRACT Grossmann G and Robertson B (Department of pediatric Pathology Karolinska sjukhuset Stockholm Sweden and Department of Pathology The Hospital for Sick Children Toronto Canada) Lung expansion and the formation of the alveolar lining layer in the fullterm newborn rabbit Acta Paediatr Scand 64 7 1975.—Light and electron microscopic studies of lungs from fullterm newborn rabbits 0–24 hours after birth revealed a patchy alveolar air expansion during the first few hours of extra uterine life Fairly uniform aeration of alveoli was noted 6–24 hours after birth but minor unexpanded areas were still present after 24 hours In the fetal pulmonary fluid as well as in the alveolar lining layer formed after the onset of breathing there are multiple large phospholipid complexes and a discontinuous multilamellar surface film can be demonstrated in some alveoli from 2 hours after birth Apparently the neonatal adaptation of the rabbit lung is a protracted process not even complete at the age of 24 hours.

KEY WORDS Neonatal lung adaptation fetal pulmonary fluid rabbit morphometry electron microscopy alveolar lining layer

Histologic and roentgenologic studies on the neonatal adaptation of the lung have given results which are in part contradictory

The fetal lung secretes a liquid into the alveolar spaces (2 4) it has been claimed that the lungs at term are fully expanded by this fluid and hence that the neonatal adaptation does not lead to any increase in the volume of the alveolar compartment (1) Also cineradiograms from normal newborn infants during and immediately after birth have suggested that the first few breaths lead to a complete aeration of the parenchyma without any significant increase in the volume of the lungs (9 14 19) This would require an instant resorption of the fetal pulmonary fluid within the first few breaths however lung weight recordings in newborn rabbits (3 7) as well as determinations of pulmonary lymph flow in newborn lambs (13) indicate that the postnatal resorption of pulmonary fluid takes at least a few hours

The purpose of the present investigation was to study the neonatal adaptation of the fullterm rabbit lung with histologic morphometric and electron microscopic techniques with particular reference to the pattern of aeration resorption of fetal pulmonary fluid and the differentiation of the alveolar lining layer The results serve as a basis for morphologic evaluation of lung expansion in premature rabbit fetuses in which the neonatal adaptation has been facilitated by deposition of homologous surfactant in the upper air ways before the first breath (21)

MATERIAL AND METHODS

The experiments were carried out on nearly fullterm rabbit fetuses In experiments concerning lung expansion 0–6 hours after birth the doe was killed by intra

sions complicating pyogenic meningitis *J Pediatr 57* 670 1960
3 Bhoudary A M Subdural empyema *J Neurosurg 32* 35 1970
4 Cinc I S Quinn J L & Bucy P C Mercury 197 and Tc 99 m brain scan in the diagnosis of non neoplastic intracranial lesions *J Neurosurg 27* 119 1967
5 Conway J J & Vollert J M The accuracy of radionuclide imaging in detecting pediatric dural fluid collection *Radiology 105* 77 1972
6 Cowan R J Maynard C D & Lassiter K R Tc 99 m pertechnetate brain scans in the detection of subdural hematomas *J Neurosurg 32* 30 1970
7 David R B Beiler D & Hood H Scintillation brain scanning in children *Am J Dis Child 112* 197 1966
8 Dunbar H S & Ray B S Localisation of brain tumors and other intracranial lesions with radioactive iodinated human albumin *Surg Gynecol Obstet 98* 433 1964
9 Feindel W Rovit R L & Stephens Newsham L Localisation of intracranial vascular lesions *J Neurosurg 18* 811 1961
10 Gilson A J & Gargano F P Correlation of brain scans and angiography in intracranial trauma *Am J Roentgen 94* 819 1965
11 Hirley P J Wagner H N Jr Diagnostic value of brain scanning in children *JAMA 221* 877 1972
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15 Lorentz W B Simon J L & Benua R S Brain scanning in children *JAMA 201* 5 1967
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Fig 1 Expansion patterns in histologic sections of lungs from fullterm rabbits (gestational age=30 days) Hematoxylin and eosin ×38 (*A*) Fetus killed in utero The alveoli are equal in size with wrinkled walls i e they have the configuration characteristic of the fluid filled state (*B*) Fetus killed 2 hours after delivery Some groups of alveoli are now expanded with air but large unexpanded areas still remain Air–liquid interfaces are preserved as rounded menisci in terminal airspaces (*arrows*) (*C*) Fetus killed 6 hours after delivery The air is more evenly distributed in the parenchyma but there are still some minor unexpanded areas (*arrows*) (*D*) Fetus killed 24 hours after delivery The alveoli are now more equal in size and polygonal

breathing small areas with somewhat over expanded alveoli and alveolar ducts were present in all animals together with more extensive areas of fluid filled alveoli retaining their fetal appearance Preserved air–liquid interfaces could be noticed in many conducting airways and there was evidence of air trapping in preterminal airspaces behind pillars of unresorbed fetal pulmonary fluid

Alveolar air expansion gradually became more uniform throughout the period of observation At later intervals (6–24 h) most alveoli were equal in size and polygonal However small unexpanded areas with fluid filled alveoli remained in all animals even at the age of 24 hours (Fig 1 B–D)

The *alveolar expansion index* was increased from an average of 0 46 before onset of breathing to 0 79 at the age of 15 min ($p<0$ 001) At the intervals ½–2 hours the mean values were somewhat lower (range 0 57–0 60) than the value obtained at 15 min the difference however not being statistically significant ($p>0$ 05) At later intervals (4–24 h) the mean value of the combined groups was statistically significantly higher (range 0 86–1 02) as compared with the combined groups 15 min–2 hours ($p<0$ 001) (Table 1)

Electron microscopic findings

In animals killed before the onset of breathing the alveoli were in part filled with acellular flocculent material (Fig 2 B) In many alveoli there were also conspicuous accumulations of phospholipid complexes i e pseudomyelin bodies surrounded by lattice figures (Fig 2 B) The largest of these accumulations measured 5×11 μm Granular pneumocytes were either mature or displayed some excess of glycogen in the cytoplasm They all contained *numerous* electron dense osmiophilic inclusions some of which apparently in the process of being extruded into the alveolar spaces (Fig 2 A)

Two hours after birth multiple air–liquid interfaces were demonstrated in the terminal airspaces However the aeration of the parenchyma was irregular with areas of well *expanded alveoli* alternating with fiords of unexpanded alveoli containg unresorbed fetal pulmonary fluid (Fig 3 A) In aerated alveoli with preserved air–liquid interfaces

Table 1 *Alveolar expansion index (I_a) at various intervals after delivery*

Postnatal age (hours)	No of animals	Alveolar expansion index (I_a) Range	Mean	S D
0	6	0 22–0 68	0 46	0 15
1/4	6	0 41–1 09	0 79	0 24
1/2	6	0 39–0 77	0 57	0 15
1	6	0 40–1 04	0 60	0 14
2	6	0 31–1 04	0 60	0 24
4	6	0 64–1 13	0 86	0 16
6	6	0 76–1 08	0 96	0 10
24	5	0 89–1 08	1 02	0 07

The difference between animals killed in utero and those killed after 15 min is statistically highly significant ($p<0$ 001) so is the difference between the combined groups 15 min to 2 hours and 4–24 hours ($p<0$ 001)

venous injection of 5 ml 2M KCl at 30 days less 0–2 hours after mating (full term=31±1 days mean±S D) As quickly as possible the abdomen was opened and the uterine vessels were clamped with large hemostats Animals to be studied before the onset of breathing were killed in utero by intraperitoneal injection of 0 5 ml Mebumal (sodium mebumal 60 mg/ml) Otherwise the animals were delivered by random uterine incisions and kept in cages at 30°C A separate group of animals was delivered spontaneously and brought to our laboratory at the age of 24 hours

Histologic morphometric studies

47 fetuses were used 6 at each of the intervals 0–6 hours and 5 at the age of 24 hours (Table 1) The unopened thorax was fixed in 10% formalin for one week Transverse slices were cut at the level of the cardiac ventricles and embedded in paraffin 10 μm thick microtome sections were made from these blocks and stained with hematoxylin and eosin Besides conventional histologic examination lung volume proportions were analysed with an integrating eye piece of the point counting type according to the principles outlined by Chalkley (5) The relative volume (V) of the following two compartments was determined

alveolar lumen all airspaces distal to the terminal bronchioles i e alveoli and alveolar duct spaces

parenchyma alveolar walls pulmonary blood vessels and lymphatics peribronchial and septal tissue and any other compartment of the lung which cannot be referred to as airspace

The complete transverse section of both lungs was examined in each case and the degree of alveolar expansion was calculated as follows

$$\text{alveolar expansion index } (I_a) = \frac{V_{\text{alveolar lumen}}}{V_{\text{parenchyma}}}$$

Electron microscopy

Two rabbits were studied at each of the intervals 0 ... 6 and 24 hours The trachea was tied the thorax opened and marginal pieces from each lung lobe were removed with a small hemostat The tissue blocks still clamped by the hemostat to preserve the air–liquid interface in the alveoli (10 23) were fixed overnight in 4% glutaraldehyde in 0 1 M sodium cacodylate buffer at 4 C Small pieces measuring approximately 1 mm were then cut from the immediate subpleural area of the tissue blocks rinsed in buffer and postfixed in 1% osmium tetroxide in buffer for 1 hour The small blocks were dehydrated in acetone and embedded in Vestopal W Thin sections were cut with an LKB Ultrotome or Reichert OmU2 ultramicrotome and stained with uranyl acetate and lead citrate The sections were examined with a Siemens 1A or a Philips 300 electron microscope

Lung weight recordings

32 animals were studied four at each interval 0–... hours (Table 2) The thorax was opened the main bronchi cut close to the parenchyma and the remaining mediastinal tissue removed The lungs were weighed together and the ratio between the wet weight of the lungs and the body weight (LW/BW ratio) was calculated

RESULTS

Light microscopic findings

The lungs from all animals killed *in utero* displayed the alveolar configuration that is characteristic of the fluid filled state i e the alveoli were equal in size with wrinkled walls (Fig 1A) After 15 min of regular

Table 2 *Lung weight/body weight ratio during the first 24 hours after delivery*

Postnatal age (hours)	No of animals	Lung weight/body weight (g/kg) Range	Mean	S D
0	4	22–29	25	3
1/4	4	21–32	26	4
1/2	4	21–24	22	1
1	4	19–24	21	2
2	4	13–17	16	2
4	4	13–16	14	1
6	4	15–18	15	3
24	4	11–13	12	1

The difference between the combined groups 0–15 min on one hand and the combined groups 30–120 min on the other is statistically highly significant ($p<0$ 001) as well as the difference between the combined groups ½–2 hours and 4–24 hours ($p<0$ 001)





Fig 3 Electron microphotographs from animal killed after 2 hours of regular breathing Well expanded alveoli with a sharp air–liquid interface alternate with fiords of unexpanded fluid filled alveoli (*A upper*) The alveolar lining layer is finely granular in most alveoli without marginal condensation (*A*) In other areas (*B*) phospholipid complexes accumulate close to the air–liquid interface and fragments of pseudomyelin material form a discontinuous surface film The lining layer not only covers the alveolar walls but it also separates gas bubbles trapped in terminal airspaces (*B*) Uranyl acetate lead citrate *A* and *B* ×12 800

cumulations of pseudomyelin bodies and lattice figures were found The largest of these aggregates measured 5×12 μm Marginal condensation of the lining layer into a delicate surface film was noted in some areas (Fig 4) in other terminal airspaces there was a discontinuous surface border formed by isolated rounded pseudomyelin bodies and irregular flakes or foils of the same material The thickness of these fragments varied from 10 to 70 nm In some areas serpentines of lamellar osmiophilic material apparently representing loose foils of surface film protruded from the air–liquid interface into the alveolar space This phenomenon was more frequent in animals killed after 6 hours than in those killed after 2 hours The structure of the inclusions of the granular pneumocytes was the same as in animals killed 0 or 2 hours after birth

Twenty four hours after delivery the lungs were almost completely air-expanded but there were still a few non aerated liquid filled alveoli As before the lining layer had a finely granular structure but its electron density was now slightly higher than that of plasma Liquid filled dimples of the alveolar wall contained multiple aggregates of pseu





Fig 2 Electron microphotographs from animal killed before the onset of breathing Osmiophilic inclusions are extruded from granular pneumocytes (A *arrow*) and large phospholipid complexes containing pseudomyelin bodies and lattice figures are floating in the alveolar spaces (B) The fetal pulmonary fluid is poo: preserved but there is some flocculent material in m: ginal portions of alveoli (B) Uranyl acetate lead citra' *A* ×4 800 *B* ×10 000

the electron density of the alveolar lining layer was similar to or slightly less than that of plasma The lining layer had a finely granular structure but it also contained several large aggregates of pseudomyelin bodies (the largest of these aggregates measuring 9×20 μm) which were in part surrounded by lattice figures In most areas there was no marginal condensation of the lining layer but in some alveoli isolated intact or fragmented pseudomyelin bodies were floating in the air–liquid interface forming a very irregular discontinuous surface film which varied in thickness between 10 and 40 nm (Fig 3 B) The floating pseudomyelin bodies had a distinct lamellar structure In a fe places rolled foils or serpentines of l: mellar osmiophilic material (thickness a| proximately 20 nm) protruded from the liqu surface into the alveolar space The incl sions of the granular pneumocytes had tl same structure as before the onset of breatl ing

Six hours after birth fiords of unex panded alveoli were still present (Fig 4) bu the majority of alveoli were now air expanc ed with a well preserved lining layer Th electron density of this layer was approxi mately the same as for plasma In liquid filled dimples of alveolar walls multiple ac





Fig 3 Electron microphotographs from animal killed after 2 hours of regular breathing Well expanded alveoli with a sharp air–liquid interface alternate with fiords of unexpanded fluid filled alveoli (*A upper*) The alveolar lining layer is finely granular in most alveoli without marginal condensation (*A*) In other areas (*B*) phospholipid complexes accumulate close to the air–liquid interface and fragments of pseudomyelin material form a discontinuous surface film The lining layer not only covers the alveolar walls but it also separates gas bubbles trapped in terminal airspaces (*B*) Uranyl acetate lead citrate *A* and *B* ×1? 800

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Fig 4 Electron microphotograph from animal killed at the age of 6 hours The alveolar lining layer contains multiple phospholipid complexes and in this area there is also a distinct multilamellar osmiophilic surface film The spacing between the lamellae is approximately 5 nm Uranyl acetate lead citrate *A* ×15 600 *B (inset)* Detail of central area in *A* ×54 000

domyelin bodies and lattice figures the larg est complex measuring 8×15 μm In addition fragments of lamellar osmiophilic material were noted both in the hypophase and the interface Although marginal condensation of the lining layer was still lacking in most ar eas (Fig 5 A), some alveoli had a fragment ed, or in places virtually continuous osmio philic surface film consisting of 10–40 nm thick flakes of lamellar pseudomyelin mate rial This feature was noted in liquid filled dimples as well as over flat portions of the alveolar walls (Fig 5 B) Serpentines of loose surface film were found in several al veoli (Fig 5 A) they were about as fre quent as in animals killed at the age of 6



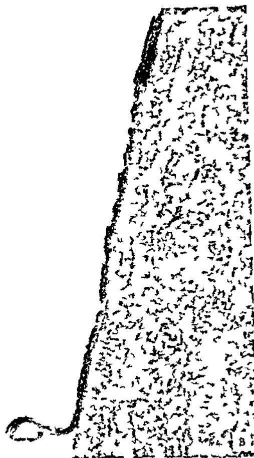


Fig 5 Electron microphotographs from animal killed at the age of 24 hours Uranyl acetate lead citrate (*A*) Air trapping in terminal airspaces is still evident and the granular lining layer surrounding the bubbles contains some phospholipid complexes Loose fragments of a surface film are indicated with *arrow* ×8 000 (*B*) In this area the alveolar lining layer has no granular hypophase the membraneous pneumocyte is coated with multilamellar osmiophilic material with a periodicity of ca 5 nm ×119 000

hours The inclusions of the granular pneumocytes had the same structure as in animals killed at earlier intervals

For all intervals it is true that the spacing of the lattice figures ranged from 20–40 nm and the periodicity of the more densely lamellated pseudomyelin material varied between 4 and 5 nm

Lung weight recordings

The lung weight/body weight ratio remained unchanged from 0–15 min (Table 2) Thereafter there was a rapid and statistically highly significant decrease up to the age of 2 hours ($p<0\ 001$) and a less prominent—although statistically highly significant—further decrease of the ratio at later intervals ($p<0\ 001$) (Table 2)

COMMENT

Our present as well as our previous results (12) lead to the conclusion that the neonatal adaptation of the fullterm rabbit lung is a process that is protracted over several hours and not even completed at the age of 24 hours This concept is at variance with statements by Aherne & Dawkins (3) and by Kikkawa et al (16) that the fullterm rabbit lung is fully aerated 1–4 hours after birth



Fig 4 Electron microphotograph from animal killed at the age of 6 hours The alveolar lining layer contains multiple phospholipid complexes and in this area there is also a distinct multilamellar osmiophilic surface film The spacing between the lamellae is approximately 5 nm Uranyl acetate lead citrate *A* ×15 600 *B (inset)* Detail of central area in *A* ×54 000

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interface However the postulated continuous surface film has not yet been demonstrated convincingly by electron microscopy This may be due to partial loss or rearrangement of the surface film during the preparation of the specimen (26) Alternatively the monomolecular film theory is an over simplification In our experimental animals the surface film was characteristically fragmented and this finding does not fit the theoretical model presented above Even where a more or less continuous surface film was observed the film was characteristically multilamellar—this would rather suggest a state of zero surface tension in those particular areas (cf 22)

The hysteresis noted in pressure/volume recordings from fullterm neonatal lungs could in part be explained by the fact that a comparatively high opening pressure is needed to force the meniscus of pulmonary fluid through the terminal bronchiole Once the alveolus has become fully aerated its diameter exceeds that of the terminal conducting airway and the pressure gradient required to maintain alveolar size is therefore less than the pressure needed to pop open the alveolus Also the residual volume normally obtained with the first few breaths might be related to air trapping in alveoli behind pillars of unresorbed fetal pulmonary fluid

For obvious reasons no morphological studies have been performed on the human lung at various stages of neonatal adaptation There is however circumstantial evidence that the adaptation of the human lung follows a course similar to the pattern we have observed in the rabbit For instance high thoracic gas volumes have been recorded in human infants shortly after birth as well as a discrepancy between thoracic gas volume and functional residual capacity This difference which disappears more rapidly in fullterm babies than in prematures indicates an initial phase of hyperexpansion of the lungs with air trapping perhaps due to the formation of stable bubbles in terminal airspaces or to bronchiolar closure (18 24) Moreover whereas the functional residual capacity of the fullterm newborn infant increases only little after 10 min of age there is a lag in the development of both maximal specific compliance and specific airway conductance indicating that the resorption of fetal pulmonary fluid is protracted for several hours also in man (for review see 20)

ACKNOWLEDGEMENTS

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REFERENCES

1 Adams F H Latta H El Salawy A & Nozaki M The expanded lung of the term fetus *J Pediatr 75* 59 1969
2 Adamson T M Brodecky V Lambert T F Maloney J E Ritchie B C & Walker A The production and composition of lung liquid in the in utero foetal lamb *In* K S Comline et al (eds) *Foetal and neonatal physiology* Cambridge University Press London 1973 p 208
3 Aherne W & Dawkins M J R The removal of fluid from the pulmonary airways after birth in the rabbit and the effect on this of prematurity and pre natal hypoxia *Biol Neonate 7* 214 1964
4 Carmel J A Friedman F & Adams F H Fetal tracheal ligation and lung development *Am J Dis Child 109* 452 1965
5 Chalkley H W Method for quantitative morphologic analysis of tissues *J Natl Cancer Inst 4* 47 1943
6 Clements J A Surface tension of lung extracts *Proc Soc Exper Med 95* 170 1957
7 deSa D J Respiratory distress accompanied by changes in the pulmonary water content and alveolar lining layer in newborn rabbits recovering from oxygen lack *Biol Neonate 13* 271 1968
8 Enhorning C & Robertson B Lung expansion in the premature rabbit fetus after tracheal deposition of surfactant *Pediatrics 50* 58 1972
9 Fawcitt J Lind J & Wegelius C The first breath A preliminary communication describing some methods of investigation of the first breath of a baby and the results obtained from them *Acta Paediatr Scand* Suppl *123* 5 1960
10 Finley T N Pratt S A Ladman A J Brewer L & McKay M B Morphological and lipid analysis of the alveolar lining material in dog lung *J Lipid Res 9* 357 1968

In the first phase of this process of adaptation (0–15 min) there is, according to our findings, a very irregular and patchy air expansion of the lungs with a tendency towards overexpansion of air filled terminal airspaces. Most alveoli remain unexpanded, i.e. filled with fetal pulmonary fluid. To judge from the unchanged LW/BW ratio the resorption of fetal pulmonary fluid is not yet initiated.

In the second phase (30 min to 2 h) there is a progressive air expansion of the lungs. The fact that this is not reflected in any change of the alveolar expansion index suggests redistribution of air within the parenchyma rather than further expansion of the total alveolar compartment. During this period the main part of the fetal pulmonary fluid is resorbed; similar observations were made by Aherne & Dawkins (3) and by deSa (7) who reported that the LW/BW ratio comes to a stable level after 2–4 hours. Large phospholipid complexes, apparently representing material extruded from the granular pneumocytes before or shortly after the onset of breathing, are present in the alveolar lining layer, but there is no distinct surface film.

During the third stage (4–24 h) the air expansion of the terminal airspaces is further improved, the configuration of alveoli becomes more uniform and polygonal, and there is some additional resorption of fluid from the lungs. The electron density of the alveolar lining layer increases and although large phospholipid complexes remain in the hypophase, some pseudomyelin bodies become fragmented or unfolded and start to generate a multilamellar film in the air–liquid interface. However, 24 hours after birth there is still no continuous osmiophilic surface film.

Phospholipid complexes apparently identical to those described in the present report were noted in the alveolar spaces of the late fetal lung also by Kikkawa et al. (15, 16) who interpreted these structures as "freely floating surfactant". Krasno et al. (17) who studied the alveolar lining layer of newborn rabbits after perfusion fixation reported a "maximum number of lamellar figures in the alveolar spaces one hour after birth" and pointed out that their findings tallied well with the peak of alveolar lecithin observed in lung wash from newborn rabbits (11). Apparently Krasno et al. (17) failed to demonstrate an alveolar air–liquid interface in animals killed before the age of 2½ hours; only their electron microphotographs from later intervals show a well demarcated alveolar lining layer comparable to that documented in our figures.

According to a commonly accepted theory, originally introduced by Clements (6), the stability of the terminal airspaces depends upon the presence of a monomolecular film of surfactant phospholipids (mainly dipalmitoyl lecithin) in the air–liquid interface. Such a phospholipid monolayer has an equilibrium surface tension of 24 dyn/cm (25). The theory implies that when surface area is compressed the phospholipid molecules become more closely packed and surface tension thereby reduced to nearly zero. Moreover, when surface area is expanded from the state of equilibrium, the distance between the surfactant molecules in the surface film increases with a consequent increase in surface tension. These deviations from equilibrium surface tension during a cyclic change in surface area is to some extent compensated for by an exchange of surfactant molecules between hypophase and interface—a process which lags behind the periods of cycling and which could explain the characteristic hysteresis noted in Wilhelmy balance recordings from lung extracts as well as in pressure/volume recordings from intact lungs.

It is usually accepted that this mechanism is operating already at the onset of breathing and that, in other words, the residual capacity normally obtained with the first breath is due to a stabilizing monomolecular film of surfactant phospholipids in the air–liquid

Acta Pædiatr Scand 64 17–24 1975

OXIDATION OF GLUCOSE AND D B OH BUTYRATE BY THE EARLY HUMAN FETAL BRAIN

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ABSTRACT Adam P A J Raiha N Rahiala E L and Kekomaki M (Departments of Pediatrics Case Western Reserve University at Cleveland Metropolitan General Hospital Cleveland Ohio USA and the University of Helsinki at the Children s Hospital Helsinki Finland) Oxidation of glucose and D-B-OH butyrate by the early human fetal brain Acta Paediatr Scand 64 17 1975.—The isolated brains of 12 previable human fetuses obtained at 12 to 21 weeks gestation were perfused through the interval carotid artery with glucose (3 mM) and/or DL-B-OH butyrate (DL-BOHB) 4 5 mM plus tracer quantities of either glucose-6-^{14}C (G6^{14}C) or β-OH butyrate-3-^{14}C (BOHB3 C) Oxidative metabolism was demonstrated by serial collection of gaseous $^{14}CO_2$ from the closed perfusion system and from the recirculating medium Glucose and BOHB were utilized at physiological rates as indicated (mean ±SEM) G6 ^{14}C at 0 10±0 01 μmoles/min g brain (n=7) or 17 5±1 9 μmoles/min kg fetus and BOHB3^{14}C at 0 16±0 05 μmoles/min g (n=5) or 27.3±7 4 μmoles/min kg

Based on fetal weight glucose metabolism by brain apparently accounted for about 1/3 of basal glucose utilization in the fetus On a molar basis BOHB3 ^{14}C was taken up at 1 47 times the rate of G6 ^{14}C Both BOHB3^{14}C and G6 ^{14}C were converted to CO_2 The rate of BOHB3 C conversion to CO_2 was equal to its rate of consumption and exceeded the conversion of glucose to CO_2 because 45% of the G6 ^{14}C was incorporated into lactate- C Accordingly both substrates support oxidative metabolism by brain and BOHB is a major potential alternate fuel which can *replace glucose* early in human development

KEY WORDS Fetus brain metabolism glucose ketone-bodies *β hydroxybutyrate*

Within the past decade many of the physiological relations between glucose and fat metabolism have been defined in the post prandial fasting or starved man Based on the arterial jugular venous gradients of B OH butyrate and the cerebral blood flow D B OH butyrate replaces glucose partially as the substrate for oxidative brain metabolism during prolonged starvation in adult man (12) More recently Persson and his co workers (17) have demonstrated similar arterial venous gradients in children between 6 weeks and 12 years of age implying that the potential to substitute B OH butyrate for glucose develops early in life In addition Page & Williamson (14) have demonstrated the presence in human brain of enzymatic activities essential to the mitochondrial uptake and metabolism of B OH butyrate

This report concerns the utilization and oxidation of both B OH butyrate and glucose by isolated perfused human fetal brain early in gestation The uptake and oxidation of glucose were examined at its usual physiological concentration in human fetal plasma

11 Gluck L Landowne R A & Kulovich M V Biochemical development of surface activity in mammalian lung III Structural changes in lung lecithin during development of the rabbit fetus and newborn *Pediatr Res 4* 352 1970
12 Grossmann G Lamke B & Robertson B Lung aeration in the fullterm newborn rabbit Roentgenologic and histologic investigations *Acta Radiol* (in press)
13 Humphreys P W Normand I C S Reynolds E O R & Strang L B Pulmonary lymph flow and the uptake of liquid from the lungs of the lamb at the start of breathing *J Physiol 193* 1 1967
14 Karlberg P The adaptive changes in the immediate neonatal period with particular reference to respiration *J Pediatr 56* 585 1960
15 Kikkawa Y Motoyama F K & Cook C D The ultrastructure of the lungs of lambs The relation of osmiophilic inclusions and alveolar lining layer to fetal maturation and experimentally produced respiratory distress *Am J Pathol 47* 877 1965
16 Kikkawa Y Motoyama E K & Gluck L Study of the lungs of fetal and newborn rabbits Morphologic biochemical and surface physical development *Am J Pathol 52* 177 1968
17 Krasno J R Knelson J H & Dalldorf F G Changes in the alveolar lining with onset of breathing *Am J Pathol 66* 471 1972
18 Krauss A N & Auld P A M Pulmonary gas trapping in premature infants *Pediatr Res 5* 10 1971
19 Lind J Tähti E & Hirvensalo M Roentgenologic studies of the size of the lungs of the newborn baby before and after aeration *Ann Paediatr Fenn 12* 20 1966
20 Nelson N M Neonatal pulmonary function *Pediatr Clin North Am 13* 769 1966
21 Robertson B & Enhorning G The alveolar lining of the premature newborn rabbit after pharyngeal deposition of surfactant *Lab Invest 31* 54 1974
22 Scarpelli E M Physiology and pathology of pulmonary surfactants *Triangle 10* 47 1971
23 Stockinger L & Hofler H Praparations und Fixationsabhangigkeit der Alveolarstrukturen *Anat Anz 127* 200 1970
24 Thibeault D W Wong M D & Auld P A M Thoracic gas volume changes in premature infants *Pediatrics 40* 403 1967
25 Tierney D F Pulmonary surfactant in health and disease *Dis Chest 47* 247 1965
26 Weibel E R & Gil J Electron microscopic demonstration of an extracellular duplex lining layer of alveoli *Respir Physiol 4* 42 1968

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'able 1 *Initial concentration of substrates and tracers*

Number of studies	Substrate concentration Glucose[a] (μmoles/ml)	DL B OH butyrate[b] (μmoles/ml)	Tracer G6[14]C (μCi/ml)	DL BOHB3[14]C[c] (μCi/ml)
[illegible]	7 97	0	0 25	0
[illegible]	0	4 48	0	0 50
[illegible]	7 97	4 48	0 25	0
[illegible]	2 97	4 48	0	0 50

[a] Measured enzymatically with coupled hexokinase and glucose-6-phosphate dehydrogenase reactions
[b] Added to the medium as sodium DL B-OH butyrate and measured with D B-OH butyrate dehydrogenase The DL B OH butyrate 4 48 μmoles/ml is equivalent to the substrate D B-OH butyrate 2 24 μmoles/ml as measured enzymatically (Table 2)
[c] Added to the medium as tracer potassium DL B-OH butyrate 3-[14]C 0 50 μCi/ml with the exception of one study (Fig 4) in which the tracer was added at an initial concentration of 0 75 μCi/ml The usual concentration 0 50 μCi/ml represents tracer D B-OH butyrate 3 [14]C 0 25 μCi/ml

and B OH butyrate dehydrogenase purchased from Boehringer Mannheim Corp New York N Y) In determining unknown lactate the highest initial concentration in the assay solution was 0 05 mM The assay mixture for D-B OH-butyrate contained NAD 0 9 mM D-B OH butyrate dehydrogenase 0 07 U/ml and the unknown at a maximum initial concentration of 0 07 mM Standard determinations for the spectrophotometric and fluorometric assays were linear in the range of concentrations assayed

Radioactivity in glucose was separated from that incorporated into organic acids by anion exchange using the analytical grade resin AG 1X8 (BioRad Berkeley Calif) Before it was used the resin was neutralized and then maintained unbuffered at a pH of 11 by repeated washing and by adding minute quantities of NaOH solution to the wash Small columns (2×0 25 inch) of approximately 1 ml volume were utilized for the separation Following precipitation and neutralization a 00 μl aliquot of supernatant from the perfusate was added to the column and eluted serially in 0 5 ml quantities using the following sequence pH 8 0 5 ml × 5 0 02 N HCl 0 5 ml × 5 0 05 N HCl 0 5 ml × 5 All the glucose was eluted within the first five 0 5 ml aliquots Alanine lactate pyruvate or B-OH butyrate was separated completely from glucose and peaked in the 10th or 11th aliquot The chromatographic recovery of labelled glucose or organic acids was 95%

Radioactivity in glucose B-OH butyrate and lactate were determined by liquid scintillation with an external standard using the premixed commercial scintillation cocktail Scintisol (Isolab Inc Akron Ohio)

In order to determine whether labelled compounds were trapped within the fetal brain a dispersed 20% homogenate of brain in 0 3 M sucrose was disrupted using a sonic dismembranator The suspension was precipitated with 10% perchloric acid and neutralized with K_2CO_3 in order to remove protein and [14]CO_2 from the medium The radio-labelled substrates were separated from products by column chromatography as indicated above The total concentration of acid stable [14]C in the brain (dpm/g) at the end of the perfusion was less than that in the medium (dpm/ml) at the end of every study and the radioactivity remaining in the precipitate was negligible indicating that labelled compounds were not trapped or concentrated within the brain substance Since the short columns employed did not discriminate between organic acids the disappearance of B-OH butyrate 3 [14]C from the medium (measured as acid stable [14]C-organic acid) quantified complete oxidation of C_4 Thus [14]CO_2 evolution from the system could be quantified at matched time intervals by the decline of acid stable [14]C in the medium during the perfusion In order to permit comparisons between the rates of consumption and production of [14]C labelled substances the disintegration rates were determined from the count rates and all results expressed as μmoles/min g brain or μmoles/min kg fetus

CALCULATIONS

In every study the disappearance of substrate between 5 and 30 minutes was linear so that all calculations have been based on the assumption of zero order kinetics

(1) Uptake of glucose-6-[14]C or B-OH butyrate 3 [14]C

$$U = \frac{(S_5^* - S_{30}^*)\,V}{25\,(S\,A)\,W}\ (\mu\text{moles/min g brain})$$

where U = uptake of substrate radioactivity S = concentration of the radioactive substrate in dpm/ml at 5 (S_5^*) and 30 (S_{30}^*) V = volume of perfusate (ml) 25 represents the time interval in minutes S A = specific activity of the radioactive substrate (dpm/μmole) and W = the weight of the brain in grams

(2) Glucose incorporation into organic acid (expressed as equivalents of glucose)

$$Inc = \frac{(L_{30} - L_5^*)\,V}{25\,(S\,A_G)\,W}\ (\mu\text{moles/min g brain})$$

where Inc = incorporation L = concentration of radioactivity in organic acid and S A_G = specific activity of glucose

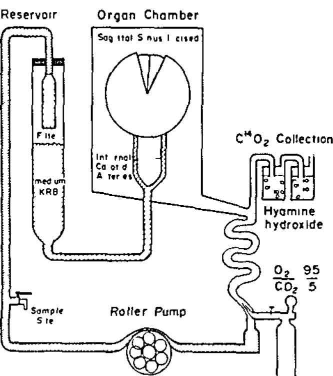


Fig 1 Schematic drawing of the closed recirculating system utilized for perfusion of isolated human fetal brain

and the uptake of D B OH butyrate was estimated at the fetal concentrations observed during prolonged maternal fasting Based on the study presented in this report the potential for oxidation of B OH butyrate by human brain develops early in fetal life

METHODS AND MATERIALS

Subjects

Human fetuses were obtained by abdominal hysterotomy from 12 pregnant women undergoing legal therapeutic abortion between 12 and 21 weeks of gestation[1] Fetal crown-rump length ranged from 8 5 to 18 0 centimeters None of the fetuses had a physical abnormality discernible during dissection

Procedure

The method of perfusion is represented schematically in Fig 1 In fetuses weighing more than 100 g (6 of 12) a polyethylene catheter was introduced into each internal carotid artery by threading from the root of the aorta and the catheters were joined with a Y connector The catheters were secured in place with surgical tape encircling the internal carotid artery In fetuses weighing less than 100 g (6 of 12) a single catheter was introduced into the aortic arch and the following vessels occluded with either surgical tape or ligature the ductus arteriosus and pulmonary arteries both subclavian arteries and both ends of the aortic arch The head was then isolated surgically from the other organs Venous return was obtained by incising the sagittal sinus which permitted the perfusate to flow through the organ chamber and to recirculate

As indicated in Fig 1 the brain was perfused with 58 5 to 93 5 ml of Krebs Ringer bicarbonate pH 7 4 37°C flowing at a rate of 8 5 ml per minute and oxygenated with a mixture of O_2 CO_2=95 5 flowing at a rate of 200 ml per minute The experimental models employed are indicated in Table 1 The glucose concentration of 2 97 µmoles/ml closely approximates the human fetal blood glucose concentration during maternal fasting and the amount of D B OH butyrate (2 24 µmoles/ml) resembles fetal levels during prolonged maternal fasting

In addition to the unlabelled substrates tracer glucose $6^{14}C$ or K DL BOH butyrate $3^{14}C$ (labelled substrates purchased from New England Nuclear Boston) was added to the medium of each study in order to evaluate the consumption and oxidation of each substrate (Table 1)

Fig 1 depicts diagrammatically the system for collection of gaseous CO_2 from the closed perfusion system The gas was bubbled through 10 ml of hyamine hydroxide in each of two bottles in series as illustrated The amount of hyamine in each bottle was a 50% excess over the total CO_2 bubbled through the bottles during each 15 minute period of infusion Since a negligible amount was collected in the second bottle only the results from the hyamine in the first bottle are reported

ANALYSES

Before enzymatic analysis or column chromatography all samples of medium were precipitated with 10% perchloric acid and neutralized with K_2CO_3 Besides precipitating the protein this procedure removed $^{14}CO_2$ quantitatively from the medium Only acid stable ^{14}C in organic compounds remained in the supernatant which subsequently was analysed enzymatically and chromatographically

Glucose was determined spectrophotometrically by coupling hexokinase and glucose 6-phosphate dehydrogenase activities (enzymes and chemicals purchased from Sigma Chemical Co St Louis Mo) The concentrations and activities in the assay mixture were ATP 2 mM $MgCl_2$ 10 mM NADP 3 3 mM hexokinase 0 95 U/ml glucose 6 phosphate dehydrogenase 0 83 U/ml and Tris buffer 0 2 M pH 7 6 In determining unknown glucose the maximum initial concentration in the assay solution was 0 2 mM

Both lactate and D B OH butyrate were determined fluorometrically in premixed glycine buffer-hydrazine solution pH 9 2 The concentrations and activities in the assay mixture for lactate were NAD 0 9 mM and lactate dehydrogenase 6 U/ml (lactate dehydrogenase

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[1] The investigators did not participate in the decision to interrupt pregnancy Studies were initiated after the fetal heart beat had ceased

Table 1 *Initial concentration of substrates and tracers*

Number of studies	Substrate concentration		Tracer	
	Glucose[a] (µmoles/ml)	DL B OH butyrate[b] (µmoles/ml)	G6[14]C (µCi/ml)	DL-BOHB3[14]C[c] (µCi/ml)
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[c] Added to the medium as tracer potassium DL B-OH-butyrate 3 [14]C 0 50 µCi/ml with the exception of one study (Fig) in which the tracer was added at an initial concentration of 0 75 µCi/ml The usual concentration 0.50 µCi/ml represents tracer D B-OH butyrate 3 [14]C 0 25 µCi/ml

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Radioactivity in glucose was separated from that incorporated into organic acids by anion exchange using the analytical grade resin AG 1X8 (BioRad Berkeley Calif) Before it was used the resin was neutralized and then maintained unbuffered at a pH of 11 by repeated washing and by adding minute quantities of NaOH solution to the wash Small columns (2×0 25 inch) of approximately 1 ml volume were utilized for the separation Following precipitation and neutralization a 200 µl aliquot of supernatant from the perfusate was added to the column and eluted serially in 0 5 ml quantities using the following sequence pH 8 0 5 ml × 5 0 02 N HCl 0 5 ml × 5 0 05 N HCl 0 5 ml × 5 All the glucose was eluted within the first five 0 5 ml aliquots Alanine lactate pyruvate or B-OH-butyrate was separated completely from glucose and peaked in the 10th or 11th aliquot The chromatographic recovery of labelled glucose or organic acids was 93 %

Radioactivity in glucose B-OH-butyrate and lactate were determined by liquid scintillation with an external standard using the premixed commercial scintillation cocktail Scintisol (Isolab Inc Akron Ohio)

In order to determine whether labelled compounds were trapped within the fetal brain a dispersed 20% homogenate of brain in 0 3 M sucrose was disrupted using a sonic dismembranator The suspension was precipitated with 10% perchloric acid and neutralized with K_2CO_3 in order to remove protein and $^{14}CO_2$ from the medium The radio-labelled substrates were separated from products by column chromatography as indicated above The total concentration of acid stable [14]C in the brain (dpm/g) at the end of the perfusion was less than that in the medium (dpm/ml) at the end of every study and the radioactivity remaining in the precipitate was negligible indicating that labelled compounds were not trapped or concentrated within the brain substance Since the short columns employed did not discriminate between organic acids the disappearance of B OH butyrate 3 [14]C from the medium (measured as acid stable [14]C-organic acid) quantified complete oxidation of C_4 Thus $^{14}CO_2$ evolution from the system could be quantified at matched time intervals by the decline of acid stable [14]C in the medium during the perfusion In order to permit comparisons between the rates of consumption and production of [14]C labelled substances the disintegration rates were determined from the count rates and all results expressed as µmoles/min g brain or µmoles/min kg fetus

CALCULATIONS

In every study the disappearance of substrate between 5 and 30 minutes was linear so that all calculations have been based on the assumption of zero order kinetics

(1) Uptake of glucose-6-[14]C or B-OH butyrate 3 [14]C

$$U^* = \frac{(S_5^* - S_{30}^*)\,V}{25\,(\mathrm{S\,A})\,W}\ (\mu\text{moles/min g brain})$$

where U = uptake of substrate radioactivity S = concentration of the radioactive substrate in dpm/ml at 5 (S_5^*) and 30 (S_{30}^*) V = volume of perfusate (ml) 25 represents the time interval in minutes S A = specific activity of the radioactive substrate (dpm/µmole) and W = the weight of the brain in grams

(2) Glucose incorporation into organic acid (expressed as equivalents of glucose)

$$\mathrm{Inc} = \frac{(L_{30}^* - L_5)\,V}{25\,(\mathrm{S\,A}_{\cdot G})\,W}\ (\mu\text{moles/min g brain})$$

where Inc = incorporation L = concentration of radioactivity in organic acid and $\mathrm{S\,A}_{\cdot G}$ = specific activity of glucose

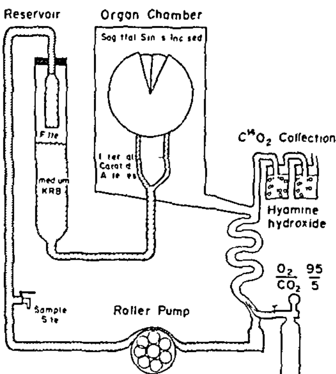


Fig 1 Schematic drawing of the closed recirculating system utilized for perfusion of isolated human fetal brain

and the uptake of D B OH butyrate was estimated at the fetal concentrations observed during prolonged maternal fasting Based on the study presented in this report the potential for oxidation of B OH butyrate by human brain develops early in fetal life

METHODS AND MATERIALS

Subjects

Human fetuses were obtained by abdominal hysterotomy from 12 pregnant women undergoing legal therapeutic abortion between 12 and 21 weeks of gestation[1] Fetal crown–rump length ranged from 8 5 to 18 0 centimeters None of the fetuses had a physical abnormality discernible during dissection

Procedure

The method of perfusion is represented schematically in Fig 1 In fetuses weighing more than 100 g (6 of 12) a polyethylene catheter was introduced into each internal carotid artery by threading from the root of the aorta and the catheters were joined with a Y connector The catheters were secured in place with surgical tape encircling the internal carotid artery In fetuses weighing less than 100 g (6 of 12) a single catheter was introduced into the aortic arch and the following vessel occluded with either surgical tape or ligature the ductus arteriosus and pulmonary arteries both subclavian arteries and both ends of the aortic arch The head was then isolated surgically from the other organs Venous return was obtained by incising the sagittal sinus which permitted the perfusate to flow through the organ chamber and to recirculate

As indicated in Fig 1 the brain was perfused with 58 5 to 93 5 ml of Krebs Ringer bicarbonate pH 7 4 37°C flowing at a rate of 8 5 ml per minute and oxygenated with a mixture of O_2 CO_2=95 5 flowing at a rate of 200 ml per minute The experimental model employed are indicated in Table 1 The glucose concentration of 2 97 μmoles/ml closely approximates the human fetal blood glucose concentration during maternal fasting and the amount of D B OH butyrate (2 2 μmoles/ml) resembles fetal levels during prolonged maternal fasting

In addition to the unlabelled substrates tracer glucose 6-^{14}C or K DL BOH butyrate 3 ^{14}C (labelled substrates purchased from New England Nuclear Boston was added to the medium of each study in order to evaluate the consumption and oxidation of each substrate (Table 1)

Fig 1 depicts diagrammatically the system for collection of gaseous CO_2 from the closed perfusion system The gas was bubbled through 10 ml of hyamine hydroxide in each of two bottles in series as illustrated The amount of hyamine in each bottle was a 50% excess over the total CO_2 bubbled through the bottles during each 15 minute period of infusion Since a negligible amount was collected in the second bottle only the results from the hyamine in the first bottle are reported

ANALYSES

Before enzymatic analysis or column chromatography all samples of medium were precipitated with 10% perchloric acid and neutralized with K_2CO_3 Besides precipitating the protein this procedure removed $^{14}CO_2$ quantitatively from the medium Only acid stable ^{14}C in organic compounds remained in the supernatant which subsequently was analysed enzymatically and chromatographically

Glucose was determined spectrophotometrically by coupling hexokinase and glucose 6-phosphate-dehydrogenase activities (enzymes and chemicals purchased from Sigma Chemical Co St Louis Mo) The concentrations and activities in the assay mixture were ATP 2 mM $MgCl_2$ 10 mM NADP 3 3 mM hexokinase 0 95 U/ml glucose 6 phosphate dehydrogenase 0 83 U/ml and Tris buffer 0 2 M pH 7 6 In determining unknown glucose the maximum initial concentration in the assay solution was 0 2 mM

Both lactate and D B OH butyrate were determined fluorometrically in premixed glycine buffer–hydrazine solution pH 9 2 The concentrations and activities in the assay mixture for lactate were NAD 0 9 mM and lactate dehydrogenase 6 U/ml (lactate dehydrogenase

[1] The investigators did not participate in the decision to interrupt pregnancy Studies were initiated after the fetal heart beat had ceased

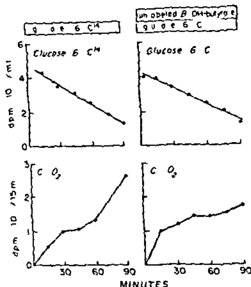


Fig 3 Effect of unlabelled B-OH butyrate on the uptake of glucose-6-¹⁴C and its conversion to $^{14}CO_2$ Supplemental sodium DL B OH butyrate (4 48 µmole/ml) was added to the medium of the study on the right

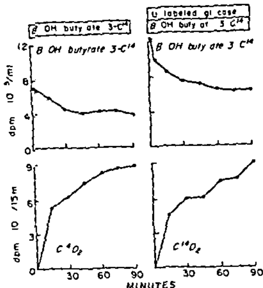


Fig 4 Conversion of B-OH butyrate 3 ¹⁴C to CO_2 in the presence and absence of unlabelled glucose The initial concentration of sodim DL B OH butyrate in the medium was 4 48 µmole /ml in both studies In the perfusion medium of the study represented on the left the initial concentration of tracer potassium DL B OH butyrate 3 ¹⁴C was 0 25 µCi/ml and the initial concentration of the labelled tracer was 0 50 µCi/ml on the right Supplemental glucose (? 97 µmoles/ml) was added to the perfusion medium of the study represented on the right

Metabolism of B OH butyrate 3 ¹⁴C

As was observed with glucose-6 ¹⁴C labelled B OH butyrate including its residual metabolites disappeared linearly from the medium during the early phase of perfusion between 5 and 30 minutes and gaseous $^{14}CO_2$ evolved continuously throughout the study (Fig 4) The disappearance of B OH butyrate differed from that of glucose however by tending to plateau shortly after 30 minutes of perfusion Consequently the difference between the initial and final concentrations of D B OH butyrate in medium (Table 2) was less than that of glucose which had declined continuously at a constant rate

The specific activity of BOHB3¹⁴C was constant throughout the study period Since the chromatographic method of separation detected the concentration of acid stable ¹⁴C in the medium and since negligible quantities of B OH butyrate 3 ¹⁴C were converted to non polar ¹⁴C the calculated rates of substrate uptake and of $^{14}CO_2$ production were identical Addition of unlabelled glucose to the medium did not modify the disappearance of B OH butyrate 3 ¹⁴C

Table 3 *Rates of substrate uptake and conversion to products (Mean ± S E M)*

Substrate	Rate measured[a]	µmoles/ min g brain
Glucose-6 ¹⁴C (n=7)	Glucose uptake[b]	0 107 ± 0 030
	G6 C uptake[c]	0 104 ± 0 013
	Lactate production	0 047 ± 0 012
	CO_2 production[d]	0 057 ± 0 011
BOHB3 ¹⁴C (n=5)	BOHB3 C uptake	0 157 ± 0 051
	$^{14}CO_2$ production	0 157 ± 0 051

Uptake and production rates between 5 and 30 of the perfusion studies

Measured enzymatically

Uptake and production of labelled substances besides $^{14}CO_2$ measured using column chromatography to separate substrates and products (see Methods)

[d] Measured by clearance of acid stable C from the medium (see Calculations)

Since 85% of the chromatographically-determined labelled organic acid was accounted for by the enzymatically measured lactate the incorporation of G6^{14}C into organic acid is referred to as the lactate production rate in order to simplify the presentation of the data

(3) $^{14}CO_2$ production

$$C^*O_2 = \frac{(C_5^* - C_{30}^*)\,V}{25\,(S\,A)\,W}\ (\mu\text{moles/min g brain})$$

where C^*O_2 = the rate of $^{14}CO_2$ production from labelled substrate C^* = the total acid stable ^{14}C concentration in the medium (dpm/ml) at 5 (C_5^*) and 30 (C_{30}^*) and S A = the specific activity of the substrate (dpm/μmole)

(4) Uptake of glucose

$$U_G = \frac{(G_5 - G_{30})\,V}{25\,W}\ (\mu\text{moles/min g brain})$$

where U_G = uptake of glucose G = the concentration of glucose in the medium (μmoles/ml) at 30 (G_{30}) and 5 (G_5)

The results were calculated on the basis of brain weight (μmoles/min g) for all 12 studies In nine of these studies the total fetal weight was measured and the results also presented on the basis of the fetal weight (μmoles/min kg)

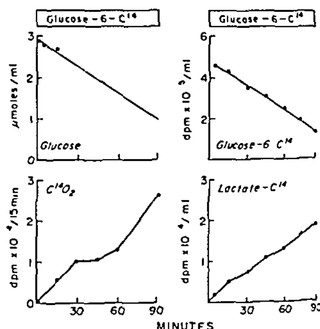


Fig 2 Conversion of glucose-6 ^{14}C to $^{14}CO_2$ and lactate ^{14}C in one representative isolated perfused human fetal brain Glucose was added to the medium at an initial concentration of 2 97 μmoles/ml and tracer glucose-6-^{14}C at an initial concentration of 0 25 μCi/ml

RESULTS

Metabolism of glucose-6 ^{14}C

A representative experiment is illustrated in Fig 2 As indicated both glucose and glucose 6 ^{14}C disappeared in a linear fashion from 5 minutes onwards throughout the range of glucose concentrations between 3 and 1 μmoles/ml (Table 2) Both lactate ^{14}C and $^{14}CO_2$ were produced continuously throughout this period (Fig 2) Labelled lactate usually accumulated linearly throughout the perfusion The amount of $^{14}CO_2$ collected usually rose during each 15 minute period of perfusion Presumably this rising rate of $^{14}CO_2$collection represented progressive ^{14}C-enrichment by exchange of the HCO_3^- pool in the medium

Glucose uptake was relatively constant and was not altered in a predictable direction by addition of B OH butyrate to the medium (Fig 3) Since the calculated rates of uptake based on weight did not vary with gestational age the results from all the studies in which the medium contained glucose-6 ^{14}C are pooled and reported in Table 3

In the seven studies of glucose 6 ^{14}C uptake the rate of uptake was 0 10 ± 0 01 μmoles/min g brain during the interval between 5 and 30 minutes of the perfusion Forty five percent of the uptake was converted to lactate and 55% to $^{14}CO_2$ This rate of $^{14}CO_2$ production would have required an oxygen consumption rate of 0 35 μmoles/min g brain

Table 2 *Initial and final concentrations of glucose lactate and B OH butyrate (μmoles/ml)*

Substance	(n)	Initial (0)	Final (90)
Glucose[a]	(9)	2 97±0 05	0 99±0 15
Lactate[b]	(9)	0	1 53±0 18
DBOHB[a]	(9)	2 24±0 07	1 60±0 07

[a] Measured enzymatically as indicated in Table 1 (Mean ± S E M)
[b] Measured with lactate dehydrogenase

and in the brain of the human fetus at 32 weeks of gestation (14)

The only attempt to quantify ketone body consumption by the human fetus *in situ* has been that of Sabata and his associates (18) They measured the umbilical V A ketone body gradients and detected a small mean umbilical venous arterial gradient of 0 05 μmoles/ml Calculation of actual fetal ketone body consumption from these data would depend on the accuracy of an assumption that fetal liver is not producing ketone bodies even though production of acetoacetate has been demonstrated in human fetal liver slices (19) On the basis of an assumed blood flow of 80 ml/kg min and the mean umbilical V A difference of 0 05 μmole/ml observed by Sabata however the apparent ketone body consumption supplied 2 5 Cal/kg 24 hrs In contrast glucose supplied 39 Cal/kg 24 hours Based on the relatively low maternal and fetal levels of ketone bodies the most likely explanation appears to be that there was sufficient glucose to suppress maternal or fetal production of ketones hence ketone body utilization may have been low merely because the fetal levels were low

III *Physiological significance of glucose and ketone body oxidation by human fetal brain*

Initially the normal fetal environment was visualized as one of abundance provided with an unlimited supply of substrates from maternal sources and buffered by maternal homeostatic mechanisms More recently a body of information has accumulated which reveals homeostatic responses of the human and mammalian fetus to temporary privation *in utero* Villee et al (20) demonstrated acceleration of anaerobic glycolysis in human fetal tissues including brain subjected to hypoxia *in vitro* This response has been confirmed in another species (9)

Goodner et al demonstrated glucose production by the rat fetus during maternal fasting (7) or hypoglycemia (6) In support of the concept that human fetuses also may produce glucose Adam et al have demonstrated that the isolated perfused human fetal liver early in gestation may produce glucose at a rate of 54 μmoles/min kg fetus for a short time (3) Similarly Chlebowski & Adam showed that the isolated canine fetal liver at term produces glucose for a more prolonged period (4) Thus human fetal brain may be protected during temporary privation by anaerobic glycolysis and fetal hepatic gluclose production

Finally the present data concerning B OH butyrate utilization by human fetal brain demonstrate that the human fetus develops the potential early in gestation to replace substrate glucose with B OH butyrate in support of oxidative metabolism Based on current understanding of B OH butyrate metabolism either maternofetal diffusion (10) or fetal production (19) could serve as potential sources for the B OH butyrate required to provide an alternative substrate for oxidative metabolism

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This work was presented in part at the annual meeting of the Society for Pediatric Research San Francisco California May 1973 A portion of the work also appeared as an abstract in *Clinical Research* 21 614 1973

REFERENCES

1 Adam P A J Control of glucose metabolism in the human fetus and newborn infant *Adv Metab Disord* 5 183 1971
2 Glucose and fat metabolism in the newborn infant of low birth weight Presented at NICHHD Conference on Intravenous Nutrition of High Risk Infants (Belmont October 26 1971) In press (1974)

and the evolution of $^{14}CO_2$ in a predictable direction and the results did not vary with gestational age, therefore the results of all studies in which the medium contained B OH butyrate 3 ^{14}C are pooled and reported in Table 3

The B OH butyrate 3 ^{14}C including intermediary aceto acetate 3 ^{14}C disappeared from the medium at approximately 1 5 times the rate of glucose 6 ^{14}C but the calculated rates of CO production and O_2 consumption were twice that obtained from glucose 6 ^{14}C This apparent discrepancy occurred because a substantial proportion of the glucose was converted to lactate

DISCUSSION

I *The isolated human fetal brain*

(1) *Quantification of glucose uptake* Quantification of glucose uptake by the human fetus and newborn infant has been inferential and based to a large extent on assumed blood flow or assumed changes in hepatic glucose output Adam has reviewed these data and has evaluated the rate of glucose production and utilization in the newborn infant (1 2) During a fast glucose production and utilization range from 35–55 μmoles/min g body weight More recently data have been obtained which indicate that the potential for basal glucose production in the mid term fetus is of the same order of magnitude as in the newborn (3) If these data represent normal physiology the rate of glucose uptake by the isolated human fetal brain at a physiological glucose concentration could account for approximately one third of total fetal glucose uptake (Table 4)

(2) *Quantification of D B OH butyrate utilization* In order to determine whether D B OH butyrate potentially could substitute for glucose early in gestation D B OH butyrate was added to the medium at a concentration in the physiological range for the fetus, according to the levels measured in amniotic fluid during the 2nd trimester (10) As indicated in Fig 4 and Table 3 B OH butyrate 3 ^{14}C disappeared from the medium and was converted to $^{14}CO_2$ In order to avoid estimating the spontaneous decarboxylation of acetoacetate to acetone the B OH butyrate in the medium was labelled on C_3 permitting evaluation of oxidation involving the tricarboxylic acid cycle The results indicate therefore that the human fetal brain oxidized B OH butyrate to CO_2

Table 4 *Magnitude of fetal cerebral glucose and D B OH hydroxybutyrate metabolism*

Substrate	(*n*)	Substrate uptake (μmoles/min kg fetus)
Glucose-6-^{14}C	(5)	17 5±1 9
BOHB3^{14}C	(4)	27 3±7 4

Mean ± S F M

In Table 4 the consumption of glucose and B OH butyrate is calculated on the basis of the fetal weight The molar consumption of B OH butyrate by the isolated brain was 1 47 times that of glucose and could account for approximately one third of energy consumed by the fetus Thus these results demonstrate that B OH butyrate potentially may provide a large proportion of the fuel for oxidative metabolism by the human fetus and the human fetal brain

II *Fetal utilization of ketone bodies*

Although the physiologic and enzymatic data supporting ketone body consumption by the human fetus are scanty there are observations which support a potential role for the ketone bodies in maintaining fetal homeostasis Ketone bodies apparently diffuse from mother to fetus *in vivo* (10 15 18) and also may be produced by isolated human fetal liver slices *in vitro* (19) In addition the essential enzymatic activities for ketone body oxidation are present in muscle kidney and brain of the suckling rat (8 9 11 13)

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TREATMENT OF MENINGITIS AND SEPTICEMIA IN INFANCY WITH A SULPHAMETHOXAZOLE/TRIMETHOPRIM COMBINATION

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From the Department of Paediatrics and the Department of Medical Microbiology University of Goteborg Goteborg Sweden

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KEY WORDS Neonatal meningitis meningitis of infants septicemia sulphamethoxazole trimethoprim

The treatment of septicemia and bacterial meningitis of infants often gives discouraging results especially when due to coliform bacteria in the neonatal period (22 10) In this age group persistance of bacteria in the cerebrospinal fluid (CSF) for long periods of time during antibiotic therapy is not unusual (15) In order to improve the survival rate and lower the number of sequelae part of the program must be studies on the effect of new antibiotics and chemotherapeutics The combination of sulphamethoxazole (SMZ) and trimetoprim (TMP) has recently been found to act synergistically and to be bactericidal to a broad spectrum of bacteria and has been an advance in the therapy of a variety of infections (1 9) Treatment of septicemia and meningitis with SMZ–TMP has been reported (4 24) including some cases in the pediatric age group (4 17 24) Experience with this combination in the first month of age is lacking

The aim of the investigation was to determine if a solution of SMZ–TMP for parenteral use could be effective in infants with septicemia and meningitis considered to be therapeutic failures after conventional antibiotic therapy The clinical and bacteriological data were to be correlated to the concentration of SMZ–TMP in serum and to the penetration into the CSF Close observation of undue effects on bone marrow blood

3 Adam P A J Glazer G Rogoff F Schwartz A L Rahiala E L & Kekomaki M Autoregulation and evolution of glucagon control of hepatic glucose production in the human fetus and canine newborn *Clin Res 20* 539 1972
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9 Itoh T & Quastel J H Acetoacetate metabolism in infant and adult rat brain in vitro *Biochem J 116* 641 1970
10 Kim Y J & Felig P Maternal and amniotic fluid substrate levels during caloric deprivation in human pregnancy *Metabolism 21* 507 1972
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TREATMENT OF MENINGITIS AND SEPTICEMIA IN INFANCY WITH A SULPHAMETHOXAZOLE/TRIMETHOPRIM COMBINATION

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ABSTRACT Sabel K -G and Brandberg Å (Department of Paediatrics and Department of Medical Microbiology University of Goteborg Sweden) Treatment of meningitis and septicemia in infancy with a sulphamethoxazole/trimethoprim combination Acta Paediatr Scand 64 25 1975 —Ten infants 8 days to 10 months old with meningitis and/or septicemia were considered therapeutic failures after conventional antibiotic treatment (i e kanamycin ampicillin and sulfonamides) and given sulphamethoxazole and trimethoprim parenterally Nine patients recovered 8 of them rapidly and one after prolonged treatment for 34 days when kanamycin was added to the combination One infant improved but later died of complications not related to the treatment High concentrations in serum and cerebrospinal fluid were achieved with a daily dose of 30–40 mg sulphamethoxazole and 6–8 mg trimethoprim per kg without signs of accumulation No change in resistance of the bacteria isolated was seen A hemolytic reaction probably due to the propylene glycol in the solution was seen in one case Other possible side effects in this age-group are discussed The antibiotic combination used seems to be a good alternative in the therapy of bacterial meningitis of infants caused by gram negative bacteria However it should still not be given to icteric or very immature infants and probably not during the first week of life

KEY WORDS Neonatal meningitis meningitis of infants septicemia sulphamethoxazole-trimethoprim

The treatment of septicemia and bacterial meningitis of infants often gives discouraging results especially when due to coliform bacteria in the neonatal period (22 10) In this age group persistance of bacteria in the cerebrospinal fluid (CSF) for long periods of time during antibiotic therapy is not unusual (15) In order to improve the survival rate and lower the number of sequelae part of the program must be studies on the effect of new antibiotics and chemotherapeutics The combination of sulphamethoxazole (SMZ) and trimetoprim (TMP) has recently been found to act synergistically and to be bactericidal to a broad spectrum of bacteria and has been an advance in the therapy of a variety of infections (1 9) Treatment of septicemia and meningitis with SMZ–TMP has been reported (4 24) including some cases in the pediatric age group (4 17 24) Experience with this combination in the first month of age is lacking

The aim of the investigation was to determine if a solution of SMZ–TMP for parenteral use could be effective in infants with septicemia and meningitis considered to be therapeutic failures after conventional antibiotic therapy The clinical and bacteriological data were to be correlated to the concentration of SMZ–TMP in serum and to the penetration into the CSF Close observation of undue effects on bone marrow blood

Table I. *Basal data of the patients and therapy given before SMZ-TMP treatment*

Case no.	Sex	Age mo.	Age days	Body weight	Bacteria isolated: CSF	Bacteria isolated: Blood	Complicating factors	Previous antibiotic treatment: drug[b]	Previous antibiotic treatment: days
1	F	10		8.4	*Proteus morganii*[a]	0	Hydrocephalus shunt infection	Ka Su	23 27
2	M	1		3.2	*Staph. albus* (contaminant)	0		Amp Ka Su	11 11 6
3	M	1		3.8	*E. coli* *Klebsiella* (after 17 days)	*E. coli*		Amp Ka Su	21 21 4
4	M	2		3.8	*H. influenzae*	*H. influenzae* *Staph. aureus*[a] (after 4 days)		Amp Su	4 4
5	F	6		8.1	*Staph. albus*	0	Hydrocephalus shunt infection	PcG cloxa	4 4
6	F		8	1.8	0	*E. coli*	Immaturity (twin)	Amp Ka	5 3
7	M	2		4.7	0		Cerebral abscess, brain oedema, ampicillin treated before first culture	Ce Su	14 14
8	F		21	3.9	*E. coli*	*E. coli*	Operated myelomeningocele, fistula of the lumbal region, hydrocephalus	Ka Su	7
9	M		21	3.7	*E. coli*	0	Urethral valve, bilateral hydronephrosis, azotemia	Amp Ka Su	5 5 5
10	M		8	2.8	*E. coli*[a]	*E. coli*	IgM trace only after 3 week illness	Co Su	3 3

[a] Bacteria still growing when therapy was changed to SMZ-TMP.
[b] Abbreviations used: Su=sulfonamide (sulfaisodimidine), PcG=penicillin G, cloxa=cloxacillin, Amp=ampicillin, Ce=cephalotine, Ka=kanamycin, Co=colistine.

and renal function as well as allergic manifestations were considered necessary.

MATERIAL AND METHODS

Ten infants with septicemia and/or meningitis treated at the Children's Hospital, Göteborg during February to August 1971 were considered to be therapeutic failures and were included in the study. Basal data of the patients in order of study are given in Table I. The age varied from 8 days to 10 months, 6 infants were one month old or less.

From 7 infants gram-negative rods were isolated from CSF or blood, one in addition had a *Staph. aureus* infection. *Staph. albus* was isolated from 2, but in one case was considered a contaminant. Two patients had infections of unknown etiology, one had been on ampicillin before the first CSF culture was taken. All infants had been treated with antibiotics parenterally, in 4 cases for more than 10 days, according to sensitivity determination, and all but 2 had in addition been on a sulfonamide. At the time therapy

Additional data suggesting persistent infection before change to SMZ–TMP
CSF white cells 10 400/mm³ CSF protein 1 010 mg/100 ml Pale grey colour frequent convulsions
WBC 53 000/mm CRP 30 μg/ml MSR 69 mm Pale grey colour
CSF white cells 1 470/mm³ CSF protein 114 mg/100 ml WBC 17 000/mm
CSF white cells (ventricle) 400/mm³ protein 270 mg/100 ml General irritation tense abdomen peaks of temperature
WBC 2 300/mm³ *CRP 38 μg/ml* Listless limp labile temperature Twin brother on Ampicillin died of *E. coli* septicemia
Peak of temperature CSF white cells ↗700/mm protein ↗79 mg/100 ml WBC 18 00/mm³ CRP ↗37 μg/ml
CSF white cells ↗34 000/mm³ CSF protein ↗1 010 mg/100 ml WBC 18 000/mm CRP 31 μg/ml
CSF white cells 1 700/mm CSF protein 565 mg/100 ml WBC 38 000/mm³ Frequent convulsions
CSF white cells 2 500/mm³ CSF protein 730 mg/100 ml WBC 15 000/mm³ CRP 121 μg/ml

was changed to SMZ–TMP bacteria were still cultured from 4 cases with gram negative infections and one with *Staph aureus* septicemia The other patients had negative cultures but infection was not under control judged from the clinical status CSF examinations white cell count and C reactive protein (CRP) in serum (23) (Table 1)

General procedure

Standard laboratory methods were used unless otherwise stated A blood count including hemoglobin red cells reticulocytes total and differential white blood count and a urinalysis for protein glucose red and white cells was done before and twice a week during treatment and was continued for one or two weeks afterwards Microsedimentation rate (MSR) and blood urea nitrogen (BUN) was followed weekly CRP (19 23) was analysed once or twice weekly

A solution of sulphamethoxazole 80 mg/ml and trimethoprim 16 mg/ml in 40% propylene glycol was used (Hoffman La Roche AG Basel code no Ro 06-2580/012) Immediately before the solution was given by the intravenous route it was diluted with seven to ten volumes of normal saline Undiluted solution was used for intramuscular administration The dose usually was 30–45 mg SMZ and 6–9 mg TMP/kg b w /24 hrs divided in two doses with an interval of 12 hours for 10 days (Table 2)

Blood samples for concentration determinations were drawn 1 hour after injection of the drug and after 12 hours immediately before a new dose was given Most samples from CSF were collected 1–2 hours after injection of the drug No efforts have been made to estimate variations of concentration due to various time of sampling

Bacteriologic procedures

Bacterial species were identified by routine methods (5) Sensitivity tests were performed by a disc method according to Ericsson et al (7) In this study the breakpoint between sensitive and resistant to SMZ–TMP (in ratio 0 1) was 16 μg/ml Serologic 0 typing of *E. coli* was performed by a method previously described (14) Determination of SMZ–TMP and TMP levels in serum and CSF was performed using the agar diffusion method (6) with paper discs as diffusion center and a *Staph albus* strain (BC Goteborg) as assay organism The medium used was peptone free blood agar plates made up from placental infusion added with 0 3% NaCl 0 05% glucose 0 1% Na_2HPO_4 $2H_2O$ 5% defibrinated horse blood and approx 1 4% Portuguese agar

To be able to calculate the TMP concentration the effect of SMZ was extinguished with a surplus of para amino-benzoic acid (PABA) in the system

RESULTS

Bacteriologic considerations

The ten strains of bacteria (Table 1) were all sensitive to SMZ–TMP with synergistic effect between the two components All were sensitive to the aminoglucoside antibiotics while four of the strains were not within the therapeutic range of ampicillin

[1] The synergistic effect of the combination SMZ–TMP varies depending on the strain of bacteria tested Thus the concentration of the combination cannot be expressed in μg/ml on a weight basis but should be considered a rougher indicator of antibacterial activity

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4	M	2		3 8	*H influenzae*	*H influenzae* *Staph aureus*[a] (after 4 days)		Amp Su	4 4
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8	F		21	3 9	*E coli*	*E coli*	Operated myelomeningocele fistula of the lumbal region hydrocephalus	Ka Su	[illegible] [illegible]
9	M		21	3 7	*E coli*	0	Urethral valve bilateral hydronephrosis azotemia	Amp Ka Su	5 5 5
10	M		8	2 8	*E coli*	*E coli*	IgM trace only after 3 weeks illness	Co Su	3 3

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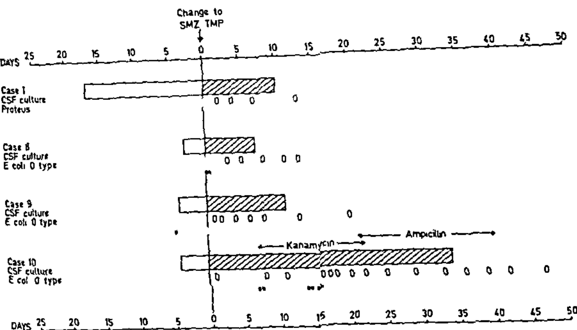


Fig 1 CSF cultures from the 4 infants who still had positive cultures at the time therapy was changed to SMZ–TMP (day 0) The previous period of antibiotic therapy is indicated by ▭ and period of parenteral SMZ–TMP by ▨ Positive cultures = + negative = 0 The results of 0 typing of the *E coli* strains are shown (not typable = ON rough form = OR)

to a rough 0 type was seen No change in resistance was observed The patient subsequently recovered after prolonged treatment with SMZ–TMP for 34 days in combination with Kanamycin and later Ampicillin (Fig 1) The patient who died (case 8) improved for 7 days on intravenous SMZ–TMP and CSF cultures subsequently were sterile but the spinal fluid still contained 800 cells/mm³ She developed progressive hydrocephalus and died of respiratory complications 3 weeks later The autopsy showed extensive signs of chronic inflammation of the spinal cord but no signs of active meningitis

Eight of the 9 survivors show normal mental development (Table 2) while one (case 1) was already severely retarded and had minor motor epilepsy before she was treated for shunt infection Eight children also show normal motor development without major sequelae Two patients have auditory defects In case 9 this was due to a toxic effect of kanamycin during a period of unnoticed renal insufficiency Case 7 who had no audiogram taken during the first year but a normal development of speech at 2 years of age unexpectedly was found to have an almost total deafness

Side effects

No side effects from skin or gastrointestinal tract were observed No local reaction was seen at the site of injection

Hemoglobin Three patients had a fall in hemoglobin of more than 2 S D during SMZ–TMP treatment and became anemic (Hb 7 3–8 5 g/100 ml) All 3 had high reticulocyte counts (140 000–200 000/mm³) These cases will be reviewed separately below

Total and differential white cell counts Two patients had eosinophils of 12% In one case this persisted for 3 weeks without any clinical signs of allergic reaction

Platelets were unaffected by SMZ–TMP

Bone marrow was aspirated in 3 cases of

Table 2 *SMZ–TMP treatment Dosage length of treatment and results*

Case no	Daily dose (mg/kg b w) SMZ	TMP	Length of treatment (days)	way of administration	Other antibiotic given	Immediate results	Follow up after 19–32 months (M 25 months) results
1	47	9 4	10	i v infusion		Recovered	Severe psychomotor retardation epilepsy
2	60	12	10	i v		Recovered	Hydrocephalus normalized after shunt operation
3	30	6	3 7	i v i m		Recovered	No sequelae
4	30	6	3 7	i v i m	Ampicillin	Recovered	No sequelae
5	30–47	6–9 4	11	i v		Recovered	Walked late strabismus
6	30	6	4 6	i v i m		Recovered	No sequelae
7	45	9	10	i v		Recovered	Late bilateral deafness
8	45–30	9–6	7	i v		Died after 3 weeks cultures negative	(See text)
9	30–43	6–8 6	11	i v		Recovered	Partial auditory defect (high tones)
10	28	5 6	32 2	i v i m	Kanamycin After 7 days ampicillin after 21 days	Recovered	Slight motoric clumsiness

Concentrations of SMZ–TMP and TMP in serum could be evaluated from 65 samples from 7 patients 8 days to 6 months old and on a daily dose of 28–47 mg/kg SMZ and 5 6–9 4 mg/kg TMP (not case 1 2 and 6) The concentration in serum 1 hour after injection mostly by the intravenous route was fairly high with a mean of 100 μg/ml (range 60–240) One case had a value of 40 μg/ml but a missed injection of the drug cannot be excluded Immediately before the next injection a mean value of 60 μg/ml was obtained (range 25–80) Concentrations were followed for more than 1 week in all cases No tendency to accumulation was noted Samples from CSF were usually drawn 1–2 hours after injection The mean value was about 1/3 of the value in serum (*M* 35 μg/ml range 25–50) The TMP concentration in serum as well as in CSF varied in a fairly constant proportion of about 1/100 of the total SMZ–TMP value A range of 0 3–1 5 μg/ml of TMP was found

Therapeutic effect and follow up

Nine of the 10 patients recovered one infant died of sequelae after 3 weeks (Table 2) All infants had sterile CSF and blood cultures after completed treatment White cells in CSF fell to less than 50 cells/mm in all cases but 2 CRP fell to zero or showed trace amounts The 4 patients with repeatedly positive CSF cultures immediately before therapy with SMZ–TMP all rapidly became negative (Fig 1) Patient no 10 however had relapses during SMZ–TMP therapy In this case the serological 0 type in the first three cultures was not typable During therapy with SMZ–TMP a change

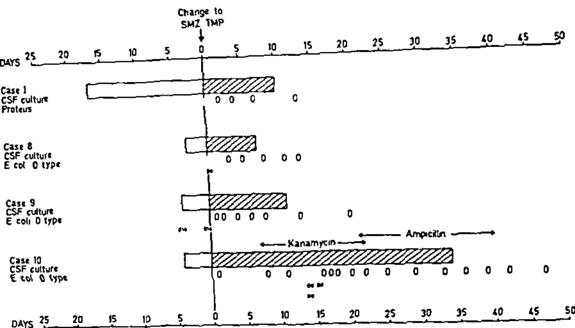


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In the single fatal case the cause of death was not directly attributed to the meningitis but to complications of a congenital hydrocephalus One patient had a relapse of the infection during SMZ–TMP and a second one during treatment with kanamycin in combination with SMZ–TMP This could not be correlated to lowered concentrations of antimicrobia in serum or CSF At 3 weeks of age only traces of IgM were found in serum while IgG and IgA were normal Later in infancy the immunoglobulins were found to be normal and the patient has not had repeated severe infections

Side effects

SMZ–TMP has not been extensively used in infants and reports of side-effects in this age group could not be found in an extensive review (1) Keuth et al (13) treated 75 infants *per os* and had only few side effects of doubtful significance Treatment for 3 months without hematological side effects has been reported (17) Disturbance of folate metabolism has been reported in malnourished children with infection during treatment with SMZ–TMP (21) and may arise in infections of long duration if the caloric supply is inadequate Leucovorin seems to eliminate these changes without interference with the antibacterial effect (11 12) We have not found any hematological changes which could be related to folate deficiency

A hemolytic reaction occurred in one case and seemed to be dose dependent Hemolysis due to sulfonamides are usually not dependent of the dose given Propylene glycol used as a solute can give hemolysis during special conditions and the manufacturer now recommends a dilution with 20–50 volumes of normal saline or 5% glucose and infusion for 1 hour

Due to the well known fact that sulfonamides may displace bilirubin from albumin binding sites thus increasing the risk of toxic effects on the CNS in an icteric infant (20) treatment during the first week of life and especially of icteric or very immature infants should still not be considered

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REFERENCES

1 Avery G S (ed) Evaluations on new drugs Trimethoprim Sulphamethoxazole *Drugs* 1 7 1971
2 Bergstrom T A prospective study of urinary tract infections in infancy and childhood Thesis Goteborg 1972 p 17
3 Brandberg Å Unpublished results
4 Calonghi G F & Ielasi G Intravenous therapy with a combination of sulphamethoxazole trimethoprim in purulent meningitis (translation) *G Mal Infett Parassit 24* 9 1972
5 Cowan S T & Steel K J *Manual for the identification of medical bacteria* Cambridge University Press London 1970
6 Ericsson H Assay of antibiotics in small amounts of fluid *Scand J Clin Lab Invest 12* 423 1960
7 Ericsson H Hogman C & Wickman K A paper disk method for determination of bacterial sensitivity to chemotherapeutic and antibiotic agents *Scand J Clin Lab Invest* Suppl *11* 21 1954
8 Fallstrom S P On the endogenous formation of carbon monoxide in full-term newborn infants *Acta Paediatr Scand* Suppl *189* 1969 *p* 19
9 Garrod L P Trimethoprim Its possible place in antibacterial therapy *Drugs 1* 3 1971
10 Gotoff S P & Behrman R E Neonatal septicemia *J Pediatr 76* 142 1970
11 Jenkins G C Hughes D T D & Hall P C A hematological study of patients receiving long term treatment with trimethoprim and sulphonamide *J Clin Pathol 23* 392 1970
12 Kahn S B Fein S A & Brodsky I Effects of trimethoprim on folate metabolism in man *Clin Pharmacol Ther* 9 550 1968
13 Keuth U Sabbagh M & Thesen E Therapie bakterieller Infektionen bei Sauglingen und Kindern *Munch Med Wochenschr 112* 802 1970
14 Lincoln K Lidin Janson G & Winberg J Resistant urinary infections resulting from changes in resistance pattern of faecal flora induced by sulphonamide and hospital environment *Br Med J III* 305 1970
15 McCracken G H Jr The rate of bacteriologic response to antimicrobial therapy in neonatal meningitis *Am J Dis Child 123* 547 1972
16 Moellering R C Jr & Fischer E G Relationship of intraventricular gentamicin levels to cure of meningitis *J Pediatr 81* 534 1972

anemia No megaloblastic changes were seen

BUN and urinalysis Patient 9 with a BUN of 88 mg/100 ml and later a diagnosis of bilateral hydronephrosis had a fall during treatment to 35 mg/100 ml All other values were normal

Review of cases with anemia

Case 9 Hemoglobin fell precipitously from 13 2 to 7 3 g/100 ml after the dose had been increased from 30 to 43 mg SMZ/kg b w /24 hrs A high carboxyhemoglobin of 2 08% in this full term infant at 4 weeks of age was a strong indication of hemolysis (8) Direct and indirect Coombs tests were negative A bone marrow aspirate showed changes in accordance with hemolysis Hemoglobin rose to 9 3 g/100 ml in less than a week after completed SMZ–TMP therapy The patient was found to have azotemia due to bilateral hydronephrosis from an uretral valve and an ureterostomy was performed

Case 10 had a slower fall over 4 3 S D to Hb 8 1 g/100 ml during long term treatment but carboxyhemoglobin was not elevated This boy had a calculated blood loss of at least 50 ml due to sampling for laboratory investigations During continued SMZ–TMP a rise was seen from 8 1 to 9 3 g/100 ml

Case 4 had a less pronounced fall of Hb from 10 8 to 8 5 g/100 ml (2 4 S D) which is not unusual during severe infection (cf 2) and was not further evaluated

DISCUSSION

Bacteriological considerations

In cases of meningitis due to *E coli* the sero types changed to rough forms Since the resistance pattern to antibiotics was unchanged this was most likely a transformation of the same *E coli* type during treatment

With the method used some antimicrobia contemporary given will interfere with the concentration determination of SMZ–TMP (i e other sulphonamides) or TMP (i e kanamycin or chloramphenicol) Only determinations without any interference of other antimicrobia are reported

In the first month of life coliform bacteria are the most common cause of purulent meningitis (22) The treatment has been penicillin G or ampicillin in the combination with one of the aminoglucosides In our study four of the seven strains of *E coli Klebsiella* and *Proteus* were resistant to ampicillin and in a larger survey (3) of 150 *E coli* strains isolated from blood cultures during 1970–71 only 17% were fully sensitive (MIC≤0 25 μg/ml) and 51% relatively sensitive (MIC≤128 μg/ml) to ampicillin By comparison TMP sensitivity was noted in 98 5% and synergism between SMZ and TMP was demonstrated in 81%

Gentamicin is now considered the drug of choice of the aminoglucosides but has a low penetration through the blood brain barrier Intrathecal administration has been used (18) but is not likely to give high intraventricular concentration (16) The concentration determinations of SMZ–TMP have shown high blood levels and good penetration into CSF even in one case with ventriculitis and minimal meningeal inflammation Penetration through the blood–brain barrier of about 1/3 to 1/4 of the concentration in serum has previously been reported in adult healthy volunteers (1)

Clinical effect

A fast recovery was seen in most cases after only a few days therapy with SMZ–TMP which is encouraging in consideration of the previously severe condition Bacteria could still be cultured from CSF in 4 of the cases when SMZ–TMP was started but cultures rapidly turned negative Other parameters such as white cells in CSF and CRP in serum (23) also suggested rapid improvement

In the single fatal case the cause of death was not directly attributed to the meningitis but to complications of a congenital hydrocephalus. One patient had a relapse of the infection during SMZ-TMP and a second one during treatment with kanamycin in combination with SMZ-TMP. This could not be correlated to lowered concentrations of antimicrobia in serum or CSF. At 3 weeks of age only traces of IgM were found in serum while IgG and IgA were normal. Later in infancy the immunoglobulins were found to be normal and the patient has not had repeated severe infections.

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9 Garrod L P Trimethoprim Its possible place in antibacterial therapy *Drugs 1* 3 1971
10 Gotoff S P & Behrman R E Neonatal septicemia *J Pediatr 76* 142 1970
11 Jenkins G C Hughes D T D & Hall P C A hematological study of patients receiving long term treatment with trimethoprim and sulphonamide *J Clin Pathol 23* 392 1970
12 Kahn S B Fein S A & Brodsky I Effects of trimethoprim on folate metabolism in man *Clin Pharmacol Ther* 9 550 1968
13 Keuth U Sabbagh M & Thesen E Therapie bakterieller Infektionen bei Sauglingen und Kindern *Munch Med Wochenschr 112* 802 1970
14 Lincoln K Lidin Janson G & Winberg J Resistant urinary infections resulting from changes in resistance pattern of faecal flora induced by sulphonamide and hospital environment *Br Med J III* 305 1970
15 McCracken G H Jr The rate of bacteriologic response to antimicrobial therapy in neonatal meningitis *Am J D s Child 123* 547 1972
16 Moellering R C Jr & Fischer E G Relationship of intraventricular gentamicin levels to cure of meningitis *J Pediatr 81* 534 1972

17 Morizaria R N Walton I G & Pickering D Neonatal meningitis treated with trimethoprim and sulphamethoxazole *Br Med J II* 511 1969
18 Newman R L & Holt R J Gentamicin in pediatrics I Report on intrathecal gentamicin *J Infect Dis 124* Suppl 1971 p 254
19 Nilsson L Å Comparative testing of precipitation methods for quantitation of C reactive protein in blood serum *Acta Pathol Microbiol Scand 73* 129 1968
20 Odell G B The dissociation of bilirubin from albumin and its clinical implication *J Pediatr 55* 268 1959
21 Postkitt E M E & Parkin J M Effect of trimethoprim sulphamethoxazole combination on folate metabolism in malnourished children *Arch Dis Childh 47* 626 1972
22 Riley H D Jr Neonatal meningitis (Editorial) *J Infect Dis 125* 420 1972
23 Sabel K G & Hansson L Å The clinical usefulness of C reactive protein (CRP) determinations in bacterial meningitis and septicemia in infancy *Acta Paediatr Scand 63* 381 1974
24 Zarouf M B M *Traitement des meningites purulentes par l association sulfamethoxazole trimethoprim* Thesis Dakar 1971

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PHYSICAL GROWTH OF 5 YEAR OLD CHILDREN WITH A LOW BIRTH WEIGHT

Stature Weight Circumference of Head and Osseous Development

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ABSTRACT Bjerre I (Department of Paediatrics Malmo General Hospital Malmo Sweden) Physical growth of 5 year-old children with a low birth weight Stature weight circumference of head and osseous development Acta Paediatr Scand 64 33 1975 —In a prospective investigation of the physical growth of children with a low birth weight 143 unselected children who weighed less than 2 500 g at birth were reviewed at 5 years of age The children were grouped according to weight relative to gestational age

Stature and weight were compared with normal curves for Swedish children and with a control series The circumference of the head was compared with a normal curve Osseous development was assessed by the method of Eklof & Ringertz and was compared with their normal values

Growth of the LBW-children was slightly retarded in respect of stature weight and osseous development but not regarding head circumference The values found for stature and osseous development were low in the group small for gestational age and for twins Those LBW-children who were appropriate for gestational age developed at a normal rate This is true even for those with the lowest birth weight and those born long before term

Osseous development was correlated with stature which may be due to the measuring method used Neither maternal disease nor feeding difficulties during the first few months of life had any demonstrable effect on the physical development of the children Socio-economic factors now surely play at most a subordinate role Physical development of the children varied most with the mothers stature and weight and probably with hereditary factors

KEY WORDS Low birth weight gestational age 5-year-olds stature body weight head circumference osseous development socio-economic factors mother s height

The physical development of children with low birth weight (LBW) has received much space in the paediatric literature But opinions still differ on the effect of LBW on further development of such children It was therefore considered warranted to undertake a prospective investigation of an unselected series of LBW children with special reference to mortality morbidity psychomotor development and socio-medical factors Physical growth during the first 5 years of life was judged from four variables viz stature weight circumference of head and osseous development The purpose was to find out whether the rate of growth differed with the type of LBW (short gestational age

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Abbreviations

LBW=low birth weight i e at most 2 500 g

AGA=appropriate for gestational age Birth weight relative to duration of gestation between the 10th and 90th percentile according to Swedish normal curves for intra uterine growth (31)

SGA=small for gestational age Weight below the 10th percentile according to the same normal curve

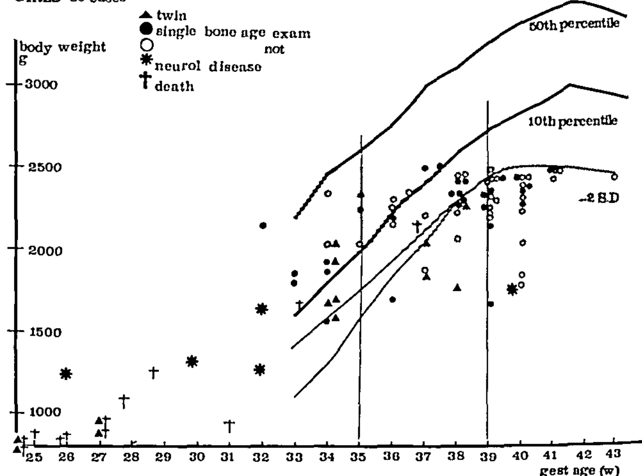


Fig 1 Distribution of the 89 girls born alive in Malmo in 1966 with LBW according to birth weight and gestational age The curve is drawn according to the normal values of Sterky (31) The upper line for −2 S D which deviates at week 37 is drawn according to a correction of the original curve made by Engstrom et al 1971 As it appears from the figure the type of classification gives only small differences in composition of groups

low weight for gestational age twin pregnancy) and to ascertain which physical dimensions are affected by retardation of growth Endeavours were also made to assess the effect of some other factors on physical growth

MATERIAL

The material consisted of all LBW children born in Malmo in 1966 The town has a population of about 250 000 inhabitants and is a well defined unit of the National Health Service It has one hospital which serves the entire population so that all the children in the town are born at one department of obstetrics and all sick children are cared for by one department of paediatrics The treatment given during the period in question was therefore uniform i e treatment of the mother during pregnancy and delivery and of the child after delivery The socio-economic standard of the people in the town is good and all inhabitants have access to good medical service All mothers and children attend prenatal clinics and children s health centres

The total number of children born alive in 1966 was 3 841 including 188 (4 9%) with LBW The total early neonatal mortality (0–6 days) was 0 89% that of LBW children 13 8% (5)

Of the 188 LBW infants 156 survived the first year None died later but 4 left the country with their parents within the 5 year period covered by the present investigation Data about these 4 children are therefore missing The remaining 152 children are living in

Table 1 *Survey of material*

	Total	AGA	SGA	Twins
Girls	70	14	46	10
Boys	73	28	33	12
Total	143	42	79	22

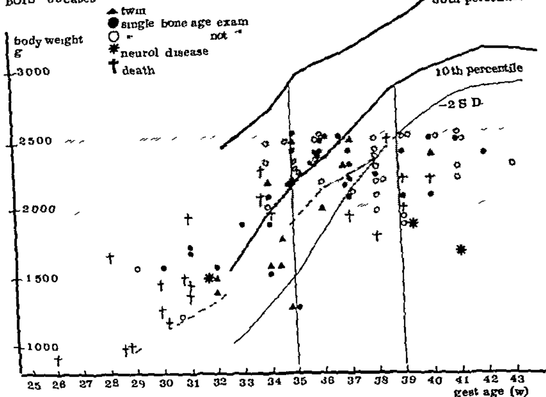


Fig Distribution of the 99 boys born alive in Malmo in 1966 with LBW according to birth weight and gestational age The curve is drawn according to the normal values of Sterky (31) The upper line for −2 S D which deviates at week 37 is drawn according to a correction of the original curve made by Engstrom et al 1971 As it appears from the figure the type of classification gives only small differences in composition of groups

Sweden and all of them were examined at 5 4±0 4 years of age

Seven of the children were excluded because of diseases capable of affecting physical growth The remaining 145 children were on the whole healthy and normally developed at 5 years of age In 2 cases the children s stature and weight were not included because they had not been correctly measured The remaining 143 children constituted 92% of those who survived the first year Notes were made of the body weight and stature of all the children the circumference of the head of 130 and osseous development was estimated in 73

Birth weight of the original 188 LBW infants in relation to gestational age is given in Figs 1 and 2 The material was divided in AGA SGA and twins (Table 1)

Head circumference was measured only in the 130 children seen personally by the author

² Only 73 children were able to come to the X ray examination They were however evenly distributed among the weight groups See also Figs 1 and 2

In some of the calculations 3 sub-groups of interest were distinguished

	Girls	Boys
1 ≤2000 g	10	14
2 ≤35th gestational week	10	12
3 >39th gestational week	31	15

Control material 50 boys and 50 girls healthy and with normal birth weight (>2500 g) picked at random from Child Health Centre in Malmö

Controls for the mothers Those women who according to the hospital records had been delivered of a child of normal body weight next after the mothers of the respective children with LBW served as controls For mothers of twins mothers of the next pair of twins were selected provided that each child weighed more than 2500 g Since twins are so often LBW the control for one of the mothers of twins had to be taken from the following year (1967)

Table 2 *Comparison of means of stature and weight differences of girls*

Group	N	Differences in stature (cm) Mean	S D	Differences in weight (kg) Mean	S D
AGA	14	+1 4	4 2	−0 5	2 0
SGA	46	−0 8***	4 3	−0 7	1 9
Twins	10	−1 7*	5 0	−1 2	1 3
≤2 000 g	10	+0 5	4 9	−0 9	2 4
≤35 w	10	+1 6	4 6	−0 5	2 4
≥39 w	31	−0 3	4 0	−0 6	2 0
Controls	50	+2 37	4 8	0 0	2 0

METHODS

Perinatal data were collected during the neonatal period Most of the children were followed up at regular intervals at the children s centre and all the children were thoroughly *examined physically* in 1971–72

Birth weight All the children were weighed to the nearest 10 g immediately after birth

Duration of pregnancy was calculated from the first day of the last menstruation When the woman was unable to give such a date with certainty the neonatal period was judged from the onset of foetal movement the size of the pregnant uterus at the first control of pregnancy by the gynaecologist and the maturity of the infant as estimated by experienced paediatricians

Age at time of review was corrected for shortfall (if any) of the period of gestation i e the child s chronological age was reduced by the number of weeks the child was born too early

Stature The stature of the children in their stockings was measured to the nearest half centimetre The recorded statures were compared with statures expected from the normal curve (8) for Swedish children and the differences found (*stature differences*) were noted for future use in the calculations

Weight The children were weighed on a platform scale with an accuracy of 0 1 kg The weight was compared with a normal curve (for Swedish boys or girls) and the *weight differences* were noted

Notes of *the mother s stature and weight* were obtained from the records of the department of obstetrics Stature was measured at the first examination at the department and the weight noted in the records was that which the mother said she had had before pregnancy

The *socio-economic distribution* of the mothers has been given elsewhere (6)

Head circumference The largest circumference was measured to the nearest 0 5 cm with a tape measure placed around the forehead and neck *The values were* compared with Nellhaus composite international normal curves (24) for girls and boys

Osseous development was appraised roentgenographically with the method of Eklof & Ringertz (13) i e measuring of 10 dimensions of the hand bones *The mean of each of the 10 variables was calculated* for each group (SGA AGA twins) and expressed as the age in years it corresponds to in a normal material (13) (unit/year) and compared with the child s age at the time of the review

Statistical methods

1 The *confidence interval* was calculated from the formula for small samples (limits of confidence interval $=\bar{x}\pm t\ s/\sqrt{n}$) In the calculation of the confidence interval for osseous development it was necessary to estimate which variables gave limits of confidence In the calculation it was necessary to exclude measurement of *the hamate bone (4 and 5)*

2 The correlation coefficient was calculated according to the formula

$$r=\frac{S_{xy}}{(n-1)\,s_x\ s_y}$$

3 Numerical differences in parameters between groups were tested with the two-sided Aspin Welch test

In the tables the significance of $0\ 01<p<0\ 05$ is denoted by one asterisk $0\ 001<p<0\ 01$ by two asterisks and $p<0\ 001$ by three asterisks

RESULTS

Stature

The mean difference in stature in the various groups is given in Tables 2 and 3

Comparison with the control group revealed as follows

1 Children who were AGA did not differ from the controls not even the children with the lowest birth weight (≤2 000 g) or those who were born long before term (≤35th week of pregnancy)

Table 3 *Comparison of means of stature and weight differences of boys*

Group	N	Differences in stature (cm) Mean	S D	Differences in weight (kg) Mean	S D
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Controls	50	+1 44	4 7	+0 6	2 0

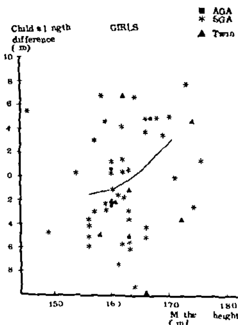


Fig 3 Correlation between stature differences of girls and heights of mothers The correlation coefficient is 0 34 *p*<0 01

2 SGA children were somewhat short The difference was statistically significant for all the girls who were SGA (*p*<0 001) but only for the SGA boys born in the 39th week of pregnancy or later (*p*<0 01)

3 The twins too were short (*p*<0 05)

No signific int difference in stature was found between h LBW groups AGA and SGA children (0 0 *p*<0 10)

Only a few children had a stature below ? S D (9 cm) so that shortness of statute was not marked (Figs 3 and 4)

On conversion of difference in stature to time and expression of the deviation of the value from the normal curve in weeks the following confidence intervals were found with a degree of confidence of 95%

Girls SGA between −16 and +4 weeks
Boys SGA between −15 and +9 weeks
Girls twins between −38 and +13 weeks
Boys twins between −45 and +2 weeks

Also expressed in this way the deviation was thus only moderate

Complications of pregnancy and delivery (only single births) occurred in 58 of the mothers (in association with pregnancy or birth of 33 boys and 25 girls) The common est complications were toxicosis of preg nancy and bleeding at parturition The aver age height of those children was not different from that of the children born after un complicated pregnancies

Feeding difficulties were noted for 16 girls and 26 boys (single births) No dif ference in stature was found at 5 years of age between children with and without feeding difficulties in their history

The differences in stature between various groups (only single births) in *social groups I+II* and *social group III* are compared in Table 4 The average length was some what smaller in social group III A signifi cant difference (*p*<0 05) was found only for the boys

Weight

The weights of all the LBW groups lay close under the standard curve No statistically

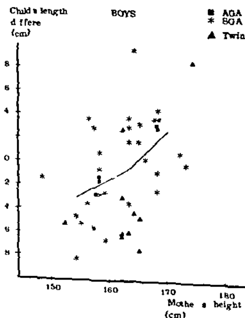


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The *socio-economic distribution* of the mothers has been given elsewhere (6)

Head circumference The largest circumference was measured to the nearest 0 5 cm with a tape measure placed around the forehead and neck The values were compared with *Nellhaus composite international normal* curves (24) for girls and boys

Osseous development was appraised roentgenographically with the method of Eklöf & Ringertz (13) i e measuring of 10 dimensions of the hand bone The mean of each of the 10 variables was calculate for each group (SGA AGA twins) and expressed the age in years it corresponds to in a normal materi (13) (unit/year) and compared with the child s age the time of the review

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≥39 w	15	−2 4 *	4 5	−0 5	1 2
Controls	50	+1 44	4 7	+0 6	2 0

Table 6 *Stature and weight of the mothers in different social groups*

The difference in the controls between stature in different social groups is statistically significant ($p<0\ 05$)

Group	N	Social groups I+II		N	Social group III	
		Mean stature (cm)	Mean weight (kg)		Mean stature (cm)	Mean weight (kg)
LBW	56	16[illegible] 4	5[illegible] 7	6[illegible]	162 3	55 2
Controls	75	165 [illegible]	56 5	77	163 3	56 6

lation coefficient was higher for boys (0 55 $p<0\ 001$) than for girls (0 34 $p<0\ 01$) and higher for AGA (boys 0 64 $p<0\ 001$) than SGA (0 45 $p<0\ 01$)

Head circumference

The observed head circumferences in all the groups corresponded to the expected mean The mean head circumference of the boys did not differ between the groups SGA AGA and twins but exceeded the expected mean given by Nellhaus (Table 7) In only a few cases did the value lie outside ±2 S D and none outside ±3 S D

Osseous development

Osseous development of the children is shown in Table 8 The confidence interval with a confidence of at least 90% expressed in weeks is given in Table 9 The values were lower for SGA than for AGA boys No such difference was found for the girls but this may be because the AGA group was very small Also the twins were few and therefore the results were difficult to interpret It was always a question of small differences from the normal material and only occasional values fell outside −2 S D The deviation in osseous development was closely correlated with deviation in stature The correlation coefficient for the entire material was 0 61 ($p<0\ 001$)

Table 7 *Mean head circumference in different groups and normal values according to Nellhaus curve (24)*

Group	N	Head circumference		Age at examination
		Mean (cm)	S D (cm)	Mean (years)
Girls				
SGA	40	50 6	1 4	5 5
AGA	13	50 7	1 [illegible]	5 2
Twins	10	50 [illegible]	1 [illegible]	5 3
All	63	50 6	1 3	5 4
Normal		50 6	1 [illegible]5	5 3
Boys				
SGA	29	51 8	1 2	5 4
AGA	[illegible]	5[illegible] 2	1 3	5 3
Twins	11	52 0	1 2	5 0
All	67	5[illegible] 0	1 2	5 3
Normal		51 4	1 25	5 3

DISCUSSION

LBW

Many authors have stressed the poor physical growth of LBW-children Especially in the earliest investigations this difference was striking (18 35) but has also been reported in more recent publications (28 32) and appears to persist throughout adult age (1) Also the children in the present investigation were mainly somewhat short and slender But the deviation from normal was small and due partly to with which values the variables studied were compared Comparison with normal curves has certain advantages but the normal curves must be valid for the population studied (27) In Sweden such normal curves are available and have been used for many years They are relatively old based on investigations made at the end of the thirties (8)

Table 4 *Comparison of differences between social groups regarding stature of mothers and of the children*

Group	Social groups I+II		Social group III	
	Children	Mothers	Children	Mothers
	Difference in stature (cm)	Stature (cm)	Difference in stature (cm)	Stature (cm)
Girls AGA	+0 70	165 5	+0 19	164 6
Girls SGA	−0 56	161 9	−1 36	162 4
Boys AGA	+2 73*	164 1	−1 18*	160 9
Boys SGA	−0 67	161 3	−1 07	161 8
All LBW girls	−0 18	162 0	−0 45	162 6
All LBW boys	+0 97*	162 4	−1 14*	161 3

significant differences were found between the groups or on comparison with the control material

When a confidential interval with 95% confidence was constructed for weight it was found that the values found for the twins and girls who were SGA were very probably below normal The confidence intervals were as follows

Girls SGA between −1 3 and −0 1 kg
Twins girls between −2 1 and 0 3 kg
Twins boys between −1 5 and −0 1 kg

As for stature the deviations were not large and only a few values fell outside −2 S D (about 3 kg)

Mother s stature and weight

The various groups differed from one another only by a few centimetres (Tables 5 and 6) The difference in mean stature between the mothers of all the LBW children and the entire control material was 2 1 cm ($p<0$ 01) The SGA mothers were on the average 1 1 cm shorter than the AGA mothers but this difference was not statistically significant Between social groups I+II and social group III no difference was found in the stature of the mothers of LBW children but in the control material the mothers in social group III were on the average 1 2 cm shorter than those in social group I+II ($p<0$ 05) The mean stature of the control material 164 4 cm was equal to that of 18 year old Swedish women 164 3 cm (8) and 20–29 year old Norwegian women 164 0 cm (22)

The differences in mean weight between the various groups were small and the range of variation within the groups was relatively wide (Tables 5 and 6) The mean weight of LBW mothers was 2 5 kg lower than that of the controls ($p<0$ 01) Weight relative to height was larger in social group III than in I+II both for LBW mothers and for the control material

In mothers of twins the same differences were found between the LBW group and the controls Only regarding weight was the difference statistically significant ($p<0$ 05)

The correlation between differences in stature of the children and the mothers heights is given in Figs 3 and 4 The corre

Table 5 *Stature and weight of mothers*

Group	*N*	Stature (cm)		Weight (cm)	
		Mean	1 S D	Mean	1 S D
AGA	41	163 0	6 1	55 5	8 1
SGA	77	161 9	5 9	53 4	7 0
All LBW single	118	162 3**	5 9	54 1*	7 5
Controls single	152	164 4**	5 8	56 6*	7 5
Twins LBW	15	163 7	5 9	53 8*	7 1
Controls twins	14	165 0	4 9	61 5*	6 8

Table 6 *Stature and weight of the mothers in different social groups*

The difference in the controls between stature in different social groups is statistically significant ($p<0.05$)

Group	N	Social groups I+II Mean stature (cm)	Social groups I+II Mean weight (kg)	N	Social group III Mean stature (cm)	Social group III Mean weight (kg)
LBW	56	162.4	52.7	62	162.3	55.2
Controls	75	165.2	56.5	77	163.3	56.6

lation coefficient was higher for boys (0.55, $p<0.001$) than for girls (0.34, $p<0.01$) and higher for AGA (boys 0.64, $p<0.001$) than SGA (0.45, $p<0.01$).

Head circumference

The observed head circumferences in all the groups corresponded to the expected mean. The mean head circumference of the boys did not differ between the groups SGA, AGA and twins, but exceeded the expected mean given by Nellhaus (Table 7). In only a few cases did the value lie outside ±2 S.D. and none outside ±3 S.D.

Osseous development

Osseous development of the children is shown in Table 8. The confidence interval with a confidence of at least 90% expressed in weeks is given in Table 9. The values were lower for SGA than for AGA boys. No such difference was found for the girls, but this may be because the AGA group was very small. Also the twins were few and therefore the results were difficult to interpret. It was always a question of small differences from the normal material and only occasional values fell outside −2 S.D. The deviation in osseous development was closely correlated with deviation in stature. The correlation coefficient for the entire material was 0.61 ($p<0.001$).

Table 7 *Mean head circumference in different groups and normal values according to Nellhaus curve (24)*

Group	N	Head circumference Mean (cm)	Head circumference S.D. (cm)	Age at examination *Mean* (years)
Girls				
SGA	40	50.6	1.4	5.5
AGA	13	50.7	1.2	5.2
Twins	10	50.5	1.2	5.3
All	63	50.6	1.3	5.4
Normal		50.6	1.25	5.3
Boys				
SGA	29	51.8	1.2	5.4
AGA	27	52.2	1.3	5.3
Twins	11	52.0	1.2	5.0
All	67	52.0	1.2	5.3
Normal		51.4	1.25	5.3

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LBW

Many authors have stressed the poor physical growth of LBW-children. Especially in the earliest investigations this difference was striking (18, 35), but has also been reported in more recent publications (28, 32) and appears to persist throughout adult age (1). Also the children in the present investigation were mainly somewhat short and slender. But the deviation from normal was small and due partly to with which values the variables studied were compared. Comparison with normal curves has certain advantages but the normal curves must be valid for the population studied (27). In Sweden such normal curves are available and have been used for many years. They are relatively old, based on investigations made at the end of the thirties (8).

Table 8 *Estimation of osseous development in years*

Group	Mean of parameter 1	2	3	4	5	6	7	8	9	10	Mean of all 10 parameters	Deviation from mean of the normal material
Girls												
AGA $n=8$	5.24	5.23	5.36	5.87	5.37	5.73	5.73	5.75	5.64	5.60	5.55	−0.11
SGA $n=20$	5.79	5.88	5.83	6.00	5.54	5.82	5.85	5.86	5.63	5.76	5.80	−0.14
Twins $n=7$	5.80	5.82	5.95	6.25	5.43	5.63	5.39	5.51	5.23	5.14	5.62	−0.27
Boys												
AGA $n=16$	5.74	5.79	5.70	6.38	5.56	5.81	5.92	6.08	5.65	5.66	5.83	+0.03
SGA $n=15$	5.27	5.57	5.73	5.93	4.95	5.61	5.63	5.46	5.08	5.26	5.45	−0.63
Twins $n=7$	6.07	5.86	6.36	6.56	5.79	5.18	5.44	5.58	4.72	4.88	5.64	+0.04

On the basis of results obtained in a comparative longitudinal study of Swedish children born 1955–58 it was concluded that the secular trend during the last 25 years had not affected growth during the first 8 years of life (14) According to other Swedish investigations from the 60s (21 29) however children now seem to be 1–2 cm taller during pre school age This agrees well with the values found in the present controls and it may therefore be concluded that LBW children are on the average somewhat shorter than normal Swedish children

Table 9 *Confidence interval with a degree of confidence of at least 90% for osseous development according to Eklof & Ringertz in LBW children*

Group	Interval	The parameters which give the limits
Girls		
AGA	−40 to 24	6 and 1
SGA	−36 to 29	6 and 1
Boys		
AGA	−25 to 36	8 and 10
SGA	−65 to 4	7 and 1

Birth weight relative to gestational age

If LBW be defined by weight relative to gestational age one fourth to one half of all of them are only small for date (34) In the present investigation more than one third of the children excluding twins and more than half of the girls were born in the 39th week of gestation or later The preponderance of girls among SGA children may be due to the fact that the limit for low birth weight was set at the same level for boys and girls though the birth weight of girls is on the average 200 g less than that of boys (31)

The mortality of SGA children is lower than that of true prematures A relatively large part of the children seen at the review were therefore SGA This probably also applies to reviews of very low birth weight children where retardation of growth has been found in some series (12 16 23 33) and in others catch up within 5–6 years (10 17 20) Besides the composition of the groups variation in early nutrition and social factors may have influenced the results

In investigations of SGA children alone shortness of stature has been reported (9 15) But also such series are heterogeneous and the results vary (26)

In agreement with the above publications the SGA children in the Malmo series were on the average short and light This was more pronounced among the boys born at term who according to the criteria also were more severely growth retarded *in utero* (Fig 2) The twins were also relatively short and light and these two groups SGA and twins lowered the average values for the entire LBW group The AGA children on the other hand did not differ from the controls This holds good even for the lightest and most preterm children and agrees well with that found in some other recent investigations of children examined in the same way but at an earlier age (2 9 17)

Head circumference

In the present material of healthy LBW children the head circumference was normal in all groups For the boys the mean value was even higher than normal This may be because the normal curve used is based on older measurements since when most boys have longer hair An increase of 1 mm in the thickness of the layer of hair means about 6 mm increase in head circumference Another contributory cause may be that pre term children often have a longer sagittal diameter of the head which results in a somewhat larger circumference (4)

A small head circumference has been regarded as implying retarded early growth of the brain and thus possibly less favour able conditions for satisfactory intellectual development (25)

Many authors have found the average head circumference of LBW children to be somewhat small both for all LBW (19 28) and for very low weight (11 33) and small for-date children (7 15)

Other authors however have claimed that the head circumference increases at a normal rate even if the child is short for age (2 35) and this agrees with the results obtained in the present material which produced no evidence of retarded growth of the skull

Osseous development

If generalised retardation of growth is due to prenatal injury one would expect osseous development too to be retarded Retarded osseous development at parturition of SGA children has thus been discussed by several author and ascribed to some intra uterine growth disorder (30) Investigations of the skeletal age of LBW-children later in life are very scanty Fitzhardinge examined small for date children at 4 years of age and found a significantly low skeletal age and a high positive correlation with growth in height (15) In the present investigation skeletal age was also somewhat lower than normal This like shortness of stature was most marked in the SGA-children and twins and a high correlation was found between retardation of osseous development and slow growth in height One might very well imagine that the dimensions obtained with Eklof & Ringertz method can vary with body height in one and the same group But Fitzhardinge used a different method (Greulich & Pyle) and obtained a similar result which suggests that the correlation between low skeletal age and shortness of stature is not ascribable to the measuring method alone It is not known whether such retarded skeletal development is of genetic origin or whether it is due to some intra uterine growth disturbance or factors in early nutrition All three factors may be contributory though they may vary in rela tive significance from one case to another

Environmental factors

Social factors probably retard growth particularly in the older series A positive correlation has been found between low social group and shortness of stature as well as between the mother s height and the frequency of LBW (3) The social dif ferences in Sweden today are surely not so

great as to influence the early nutrition of the child. In the present investigation the LBW children belonged to the same social groups as the controls (6). Differences in stature between the children in the social groups were also small and were possibly due to heredity rather than to the social class to which the child belonged.

Perinatal and genetic factors

It is very probable that there is a correlation between the degree of growth retardation *in utero* and later in life. In this study, however, this seemed not to be correlated with obstetric factors, but a more thorough study of this complex problem is in progress.

In a given case physical growth can of course depend on several factors, but the most important factors in the group as a whole seem to be the mother's height and weight. This holds good for both AGA and SGA children and appears to be independent of social class. Thus according to this study the shortness of many LBW children, particularly those who are SGA, appears to be mainly of genetic origin.

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REFERENCES

1. Alm, I.: The long-term prognosis for prematurely born children. A follow-up study of 999 premature boys born in wedlock and of 1 002 controls. *Acta Paediatr Scand* Suppl 94, 1953.
2. Babson, S. G.: Growth of low-birth-weight infants. *J Pediatr 77*: 11, 1970.
3. Barron, S. L.: The epidemiology of human pregnancy. *Proc R Soc Med 61*: 1200, 1968.
4. Baum, J. D.: Head shape and size of preterm low-birth-weight infants. *Dev Med Child Neurol 13*: 576, 1971.
5. Bjerre, I. & Östberg, G.: Infant mortality. *Acta Paediatr Scand 63*: 49, 1974.
6. Bjerre, I. & Varendh, G.: Socio-economic factors in low birth weight. (To be published.)
7. Brandt, I.: Dynamik des postnatalen Schädelwachstums bei Früh- und Reifgeborenen bis zum 18. Lebensmonat. *Monatsschr Kinderheilkd 121*: 12, 1973.
8. Broman, B., Dahlberg, G. & Lichtenstein, A.: Height and weight growth. *Acta Paediatr Scand 30*: 1, 1942.
9. Cruise, M. O.: A longitudinal study of the growth of low birth weight infants. I. Velocity and distance growth, birth to 3 years. *Pediatrics 51*: 620, 1973.
10. Dann, M., Levine, S. Z. & New, E. V.: A long-term follow-up study of small premature infants. *Pediatrics 33*: 945, 1964.
11. Davies, F. A. & Davis, J. P.: Very low birth weight and subsequent head growth. *Lancet II*: 1216, 1970.
12. Drillien, C. M.: Growth and development in a group of children of very low birth weight. *Arch Dis Child 33*: 10, 1958.
13. Eklöf, O. & Ringertz, H.: A method for assessment of skeletal maturity. *Ann Radiol 10*: 330, 1967.
14. Engström, I., Karlberg, P., Klackenberg, G., Klackenberg-Larsson, I., Lichtenstein, H., Svennberg, I. & Taranger, J.: Growth diagrams for height, weight and head circumference from birth to the age of 18 years. *Läkartidningen 70*: 2960, 1973.
15. Fitzhardinge, P. M. & Steven, E. M.: The small-for-date infant. I. Later growth patterns. *Pediatrics 49*: 671, 1972.
16. Frisk, M., Takkunen, R. L. & Holmström, G.: Small prematures at 6–7 years of age. III. Comparison of prematures born in 1955 and 1956. *Ann Paediatr Fenn 10*: 79, 1964.
17. Gairdner, D. & Pearson, J.: A growth chart for premature and other infants. *Arch Dis Child 46*: 783, 1971.
18. Illingworth, R. S.: The after-history of premature infants. *Arch Dis Child 14*: 121, 1939.
19. Justessen, K.: Cranial circumference of premature children. *Acta Paediatr Scand 51*: 13, 1962.
20. Koenig, H.: What happens to prematures. *Am J Public Health 40*: 803, 1950.
21. Köhler, L.: Physical examination of four-year-old children. *Acta Paediatr Scand 62*: 181, 1973.
22. Lindberg, W., Natvig, H., Rygh, A. & Svendsen, K.: Høyde og vegtundersøkelser hos voksne menn og kvinner. *Tidsskr Nor Laegeforen 76*: 361, 1956.
23. Lubchenko, L. O., Horner, F. A., Reed, L. H., Hix, I. E., Metcalf, D., Cohig, R., Elliott, H. C. & Bourgh, M.: Sequelae of premature birth. *Am J Dis Child 106*: 101, 1963.
24. Nellhaus, G.: Head circumference from birth to eighteen years. *Pediatrics 41*: 106, 1968.
25. O'Connell, E. J., Feldt, R. H. & Stickler, G. B.: Head circumference, mental retardation and growth failure. *Pediatrics 36*: 62, 1965.
26. Ounsted, M.: Postnatal growth of children who

were SFD or LFD *Dev Med Child Neurol 13* 171 1971

27 Pryor H B Charts of normal body measurements and revised width weight tables in graphic form *J Pediatr 68* 615 1966

28 Robinson N M & Robinson H B A follow-up study of children of low birth weight and control children at school age *Pediatrics 35* 425 1965

29 Samuelson G An epidemiological study of child health and nutrition in a northern Swedish county III Medical and anthropometrical examinations *Acta Paediatr Scand 60* 653 1971

30 Scott K E & Usher R Epiphyseal development in fetal malnutrition syndrome *N Engl J Med 270* 82 1964

31 Sterky G Swedish standard curves for intrauterine growth *Pediatrics 46* 7 1970

32 Wohlmuth G & Frater R Late sequelae of prematurity *Acta Paediatr Acad Sci Hung 6* 79 1965

33 Wright F H Blough R R Chamberlin A Ernest T Halstead W C Meier P Moore R Y Naunton R F & Newell F W A controlled follow up study of small prematures born from 1952 through 1956 *Am J Dis Child 124* 506 1972

34 Yerushalmy J van den Berg B J Erhardt C L & Jacobziner H Birth weight and gestation as indices of immaturity *Am J Dis Child 109* 43 1965

35 Ylppo A Das Wachstum der Fruhgeborenen von der Geburt bis zum Schulalter *Z Kinderheilkd 24* 111 1919

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PRENATAL DIAGNOSIS OF METHYLMALONIC ACIDURIA

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KEY WORDS Methylmalonic aciduria methylmalonic acidemia vitamin B_{12}, prenatal diagnosis

Four congenital methylmalonic acidurias each with a different etiology have now been defined (Fig 1) deficiency of methyl malonyl CoA racemase (E C 5 1 99 1) activity (4) deficiency of methylmalonyl CoA mutase (E C 5 4 99 2) activity because of a defective apoenzyme (8) and two abnormalities of vitamin B_{12} metabolism which secondarily lead to deficiency of methylmalonyl CoA mutase holoenzyme activity because of decreased availability of the necessary coenzyme 5 deoxyadenosylcobalamin (Ad B_{12}) (7) The two disorders of B_{12} metabolism produce abnormalities in the conversion of the vitamin form of B_{12} hydroxocobalamin (OH B_{12}) to the conenzyme forms Ad B_{12} and methylcobalamin (Me B_{12}) In one disorder there is failure to synthesize normal amounts of Ad B_{12} but normal synthesis of Me B_{12} while in the other neither coenzyme is made Each of the four diseases is characterized by protein intolerance attacks of ketoacidosis and failure of growth and development In the types caused by defects of vitamin B_{12} metabolism pharmacologic doses of B_{12} often produce clinical and chemical improvement (3 5)

Each of the methylmalonic acidurias is thought to be inherited as an autosomal recessive trait and theoretically ought to be detectable prenatally using cultured amniotic fluid cells One previous attempt at prenatal

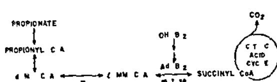


Fig 1 Metabolic path of methylmalonyl CoA to show where blocks occur in the methylmalonic acidurias and to indicate the assays used in diagnosis (see Text) Abbreviations *d* and *l* MM CoA *are d and l* methylmalonyl coenzyme A OH B 2 is hydroxocobalamin Ad B_{12} is 5 deoxyadenosylcobalamin

diagnosis has been reported by Morrow and colleagues (9) They predicted an affected fetus in the third trimester because of elevated methylmalonate in amniotic fluid and maternal urine and confirmed the diagnosis after birth Our communication reports prenatal diagnoses in two second trimester pregnancies The two pregnancies were at risk for different types of methylmalonic aciduria In one a normal fetus was diagnosed and later confirmed after birth in the other an affected fetus was predicted and confirmed by studies on the abortus

CASE REPORTS

The pregnancy was the third for family P Their first child was known to have a B responsive form of methylmalonic aciduria due to a block in the conversion of vitamin B 2 to Ad B 2 He is being successfully treated with protein restriction and high dose B 2 therapy (5) There is a younger daughter who is normal

Family E had had one previous pregnancy A girl who appeared normal at birth had metabolic acidosis at 3 days of age and recurrent vomiting and ketoacidosis thereafter When 16 months old she weighed 7 kg and had a developmental quotient of 35 Diagnosis of methylmalonic aciduria was established with a daily urine excretion of 2-2 5 g of methylmalonate She died at 18 months of age B_{1} therapy had not been tried and the type of methylmalonic aciduria in family E was undetermined

METHODS

Amniotic fluids obtained by transabdominal amniocentesis were centrifuged and the cells placed in culture Supernatal amniotic fluids and maternal urines were stored at −60° until methylmalonate assay was performed by gas liquid chromatography Methylmalonate was purified from urine and amniotic fluid by ion exchange chromatography (?) The column eluate was extracted with diethyl ether and methylmalonic acid was converted to the trimethylsilyl ethers

Three assays details of which have been previously published were used to study cultured cells The first incubated intact cells with 1 65 mM propionate 3-^{14}C methyl-^{14}C malonate or succinate 1 4- C and measured the oxidation of each substrate to CO_2 (11) (Fig 1) The second studied the conversion of ^{57}Co-OH B_{12} to the B 2 coenzymes in cells growing in culture (9) The culture medium contained 0% human serum instead of fetal calf serum to enhance the uptake of OH B 2 by the cells The third assay used cell lysates to measure methylmalonyl-CoA mutase activity by quantitating the conversion of racemic ^{3}H methylmalonyl CoA to ^{3}H succinyl-CoA in the presence of excess Ad B 2 coenzyme (1)

RESULTS

Amniotic fluid cell studies

Amniocentesis was done at 12 weeks gestation for Mrs P and at 16 weeks gestation for Mrs E The cells were studied after 4 weeks in culture They were fibroblastic from the P pregnancy and predominantly epithelioid from the E pregnancy We have used both fibroblastic and epithelioid amniotic fluid cell cultures as controls for the three assays and have found no significant difference between the cell types

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Table 2 *Accumulation of B_{12} Coenzymes in cultured amniotic fluid cells*

Control data are mean±S D from eight normal cultures

	pg coenzyme/mg cells	
	Ad B_{12}	Me B_{12}
Control Cells	1 2±0 8	1 1±0 8
P Cells	0 8 1 0	0 5 0 7
E Cells	1 5	1 1

either propionate or methylmalonate while oxidizing succinate normally Succinyl-CoA is beyond the blocks in the methylmalonic acidurias The results indicated an abnormality in the metabolism of methylmalonyl CoA by cells from the E pregnancy

Table 2 contains data for B_{12} coenzyme accumulation by cells in culture Cells were grown for four days in medium containing ^{5}Co-OH B_{12} and then the labelled intracellular coenzymes Ad B_{12} and Me B_{12} were measured This assay had been abnormal using skin fibroblasts of the affected proband in family P His cells accumulated almost no Ad B_{12} (0 01 pg/mg cells) but accumulated normal and less than 5 mg/day For Mrs E tic fluid cells from both the P and E preg nancies showed normal accumulation of both coenzymes

Direct assay of methylmalonyl-CoA mutase activity was done only with the cells from the E pregnancy Enzyme activity was severely deficient at 0 03 units[1] compared with a simultaneously run control with 2 02 units

Methylmalonate in amniotic fluid and maternal urine

Mrs P had urinary methylmalonate determined at eight week intervals throughout the pregnancy and the excretion was always normal and less than 5 mg/day For Mrs E methylmalonate was measured in amniotic fluid at the time of amniocentesis at 16 weeks and before therapeutic abortion at 2 weeks (Table 3) It was elevated on both occasions Urinary excretion of methylmalonate by Mrs E was also elevated from the 16th week until abortion and then fell rapidly back to normal

Confirmatory data

Mrs P completed her pregnancy and gave birth to a normal son In the first day of life oxidation of ^{14}C-propionate by white blood cells was normal and no methylmalonate was detected in his urine Later cultured foreskin fibroblasts were shown to have normal propionate and methylmalonate oxidation and normal accumulation of B_{12} coenzymes The E family elected to terminate the pregnancy and fetal skin fibroblasts were used to confirm the diagnosis of an affected fetus In the propionate oxidation assay fibroblasts from the E fetus made <0 001 μmoles $^{14}CO_2/10^6$ cells/3 hr compared with the control cells with 0 02 μmoles and in the assay for methylmalonyl

Table 3 *Methylmalonate content of amniotic fluid and maternal urine*

Data are from the E pregnancy and from control mid trimester pregnancies

	Methylmalonate		
Gestation stage	Amniotic fluid (μg/ml)	Urine (mg/d)	Urine (mg/g creatinine)
16 weeks	4 3	6 0	5 2
18 weeks		9 1	7 8
19½ weeks		14	11
20 weeks		10	8 5
21 weeks		11	9 2
22 weeks		13	9 3
23 weeks		12	12
Day of abortion	5 6		22
Day after abor tion		5 5	5 0
Days 2–7 after abortion		2 3–2 7	2 1–2 6
Controls (±S D)	<1 (n=8)	1 8±0 7 (n=10)	1 3±0.5 (n=10)

[1] 1 unit=1 nanomole succinate formed/mg protein/30 min

CoA mutase activity a lysate of cells from the E fetus had 0 13 units activity while the control lysate had 2 3 units

DISCUSSION

Each of the four methylmalonic acidurias has been accompanied by a severe block in propionate and methylmalonate oxidation in cultured skin fibroblasts from affected patients This suggests that the oxidation assay using cultured amniotic fluid cells has the potential for diagnosing any of the disorders *in utero* To differentiate among the four further tests would have to be done The B_{12} coenzyme assay can define the two disorders of B_{12} metabolism Based on skin fibroblast data the mutase assay should show an almost total absence of activity in the mutase apoenzyme defect and a 50% decrease in activity in the racemase deficiency when racemic methylmalonyl CoA containing a 50/50 mixture of D and L methylmalonyl CoA is used as substrate Mutase activity in the presence of excess Ad B_{12} would be normal in the two B_{12} disorders

Cells from the P pregnancy oxidized propionate and methylmalonate normally Prediction of a normal fetus was verified with studies in skin cells after birth

Cells from the E pregnancy had a greatly diminished capacity to oxidize either propionate or methylmalonate That result established that we were dealing with one of the blocks in methylmalonyl CoA metabolism Definition of the type of methylmalonic aciduria in family E had not been possible in the deceased proband and thus we needed further tests to better define the disease The normal B_{12} coenzyme study plus the severe deficiency in mutase activity indicated that the fetus had a mutase apoenzyme defect Previous patients with this type of defect have not been helped by high dose B_{12} therapy Had the fetus been affected with an error of B_{12} metabolism increased options could have been offered to the family including prenatal therapy with vitamin B_{12} Prediction of the effectiveness of prenatal or postnatal B_{12} therapy would be unwarranted however unless such therapy had been tried in a sibling four patients are now being recognized who show *in vitro* cell findings of a B_{12} metabolic error but who do not respond to *in vivo* therapy with vitamin B_{12} (10)

The finding of elevated methylmalonate in amniotic fluid and maternal urine in the E pregnancy between 16 and 23 weeks encourages us that those determinations themselves may be diagnostic in time to elect therapeutic abortion If this proves correct diagnoses could be made in a few days instead of the 4–4½ weeks currently needed for cell studies The immediate fall of urine methylmalonate to normal levels when abortion was performed suggests that the fetus was the source of the methylmalonate This is further supported by normal excretion throughout pregnancy in Mrs P who was carrying an unaffected fetus but who is a presumptive heterozygote for the mutation causing defective Ad B_{12} synthesis It is possible that a heterozygote for one of the other defects might show an increased excretion in pregnancy even with a normal fetus In the previously reported attempt at prenatal detection (9) elevation of methylmalonate in amniotic fluids and maternal urine was not definite until the third trimester too late to elect abortion Thus until further data are collected correlating studies in cultured amniotic fluid cells with methylmalonate determinations in amniotic fluid and maternal urine we believe diagnoses should be based primarily on cell studies or on a combination of cell and fluid studies

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REFERENCES

1 Cardinale G J Dreyfus P M Auld P & Abeles R H Experimental vitamin B_{12} deficiency its effect on tissue vitamin B_{12} coenzyme levels and on the metabolism of methylmalonyl-CoA *Arch Biochem Biophys 131* 92 1969
2 Giorgio A J & Plaut G W E Method for colorimetric determination of urinary methylmalonic acid in pernicious anemia *J Lab Clin Med 66* 667 1965
3 Hsia Y E Scully K Lilljeqvist A Ch & Rosenberg L E Vitamin B_{12} dependent methylmalonic aciduria *Pediatrics 46* 497 1970
4 Kang E S Snodgrass I J & Gerald P S Methylmalonyl coenzyme A racemase defect another cause of methylmalonic aciduria *Pediatr Res 6* 875 1972
5 Lindblad B Lindstrand K Svanberg B & Zetterström R The effect of cobamide coenzyme in methylmalonic acidemia *Acta Paediatr Scand 58* 178 1969
6 Mahoney M J & Rosenberg L E Synthesis of cobalamin coenzymes by human cells in tissue culture *J Lab Clin Med 78* 302 1971
7 Mahoney M J Rosenberg L E Mudd S H & Uhlendorf B W Defective metabolism of vitamin B_{12} in fibroblasts from children with methylmalonic aciduria *Biochem Biophys Res Comm 4* 375 1971
8 Morrow III G Barness L S Cardinale G J Abeles R H & Flaks J G Congenital methylmalonic acidemia enzymatic evidence for two forms of the disease *Proc Natl Acad Sci USA 63* 191 1969
9 Morrow III G Schwarz R H Hallock J A & Barness L A Prenatal detection of methylmalonic acidemia *J Pediat 77* 120 1970
10 Morrow III G (personal communication) & Mahoney M J Unpublished data
11 Rosenberg L E Lilljeqvist A Ch & Hsia Y E Methylmalonic aciduria metabolic block localization and vitamin B_{12} dependency *Science 162* 805 1968

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METHOD FOR DETERMINATION OF PULMONARY GAS EXCHANGE IN CONNECTION WITH BIRTH

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ABSTRACT Tunell R (Department of Paediatrics Karolinska Sjukhuset Stockholm Sweden) Method for determination of pulmonary gas exchange in connection to birth Acta Paediatr Scand 64 49 1975.—An apparatus built on the open system for determination of pulmonary gas exchange in the newborn infant after birth is described At four minute intervals diluted expired air (5–7 l/min) was collected in bags The oxygen and carbon dioxide fraction in the bags were analysed with a Nyons Diaferometer (working on the principle of thermoconductivity) In calibration experiments using a gas-mixing technique a high degree of linearity was found both in the determination of the fraction of oxygen and carbon dioxide ($r=0\ 9996$) Reproducibility from duplicate readings was also good (for oxygen determination 0 9% and for carbon dioxide determination 0 8) Duplicate determinations performed on infants with the same degree of motor activity resulted in an estimated error of the method of 5 8% for V_{O_2} and 7 8% for V_{CO_2} respectively A metabolic chamber was used to control environmental temperature The air temperature and wall temperature in the chamber were regulated by water from a thermostatically controlled waterbath and were kept equal within 0 5°C As the method for determination of the fraction of oxygen and carbon dioxide is not specific other gaseous materials exhaled by the infants influence the measurements and nitrous oxide was found to interfere with the determinations and made V_{O_2} and V_{CO_2} determinations in these patients impossible Experience from more than 50 investigations on newborn infants has shown that the method is well suited to this particular type of study

KEY WORDS Newborn infants oxygen consumption carbon dioxide production open circuit apparatus environmental control

At birth the human newborn infant is in a state of hypoxemia hypercarbia and metabolic acidosis referred to as asphyxia (9 14 15 29) Within 15–20 min after birth the pulmonary gas exchange has resulted in normalization of arterial blood gases and a diminishing degree of acidosis (5 19 23) Simultaneous determination of pulmonary gas exchange and blood gases after birth would enable the quantitative evaluation of the oxygen supply and the carbon dioxide elimination immediately after birth

The influence of different environmental conditions on oxygen consumption in the newborn infant has been reported in several investigations (1 13 26) Most of these investigations have started 20–30 min after birth The critical period 0–20 min after birth has been investigated sporadically and then only with isolated determinations of short duration (usually about 5 min) (16 25)

If the initial period after birth is to be investigated special demands are required from the equipment for determination of pul

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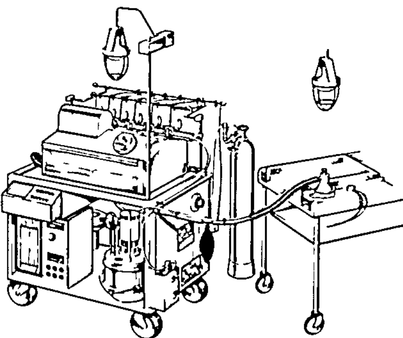


Fig 1 Equipment used in delivery room On the delivery bed Face mask open infant bed and heating lamp On the rolling table Bags for gas collection infant chamber tape recorder temperature recorder and water bath

monary gas exchange It must be possible to start the investigation at delivery within the first seconds after birth and to continue the investigation without interruption during the first 20 min after birth Because of the rapid changes in pulmonary gas exchange it is necessary to make the determinations with a minimum of lag time The equipment should also make it possible to keep the infants in predetermined environmental thermal conditions

As no such equipment is commercially available an apparatus meeting these requirements has been constructed

Short Outline of Apparatus and Handling of the Infants

Oxygen uptake and carbon dioxide elimination were determined by the open principle using a face mask and collecting the diluted expired air over 4 minute periods into bags which were subsequently analysed for their volume and gas composition

The routine care of the newborn infants including suction of upper air ways drying of the skin and clamping and dividing the umbilical cord was performed on an open infant bed placed at the delivery table

As soon as spontaneous breathing had occurred the face mask was applied and air collection was started Without interruption air collection was continued during the subsequent 20 min and then at predetermined periods As soon as possible and always within 10 min after birth the infants were transferred into a thermo controlled chamber

Throughout the investigation the infants were closely watched skin and rectal temperatures were monitored and the degree of muscular activity was assessed and recorded using a four point scale 0= asleep or awake but no movements 1=slight movements of one or two extremities 2=movements of all four extremities and 3= the infant crying and active For each period of gas collection (4 min) the mean activity was calculated

Description of apparatus

The apparatus consists of three components

(1) The respiratory air supply and collection system
(2) Equipment for environmental thermal control and temperature control
(3) Equipment for determination of respiratory gas volume and composition

Respiratory air supply and collection system

(Figs 1 2 and 3)

The face mask (Fig 3) was made up of three parts An outer double walled Plexiglas funnel with circulating water between the walls an inner single walled Plexiglas funnel and a Plexiglas ring (10 cm diameter) adjusted to a plastic cap The plastic cap lubricated with liquid paraffin covered the back of the infant s head The outer funnel fitted into the ring and could be disconnected instantaneously to give free access to the face of the infant The Plexiglas ring was held by hand and kept 1–2 cm above the face of the infant The opening of the inner funnel covered the mouth and nose of the infant

Air was supplied to the face mask from a cylinder with compressed air free of CO_2 at a flow of 5–7 l/min and was monitored by a flowmeter The air was conducted through a humidifier consisting of a copper bottle with water kept at 80°C by a heating plate (max power 50 W) regulated by a variable resistance In order to avoid pressure differencies in the system a thin walled

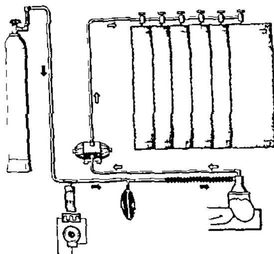


Fig 2 Respiratory air supply and collection system Cylinder with compressed air (dry and free of CO_2) humidifyer rubber bag face mask pump and bags with three way stopcocks

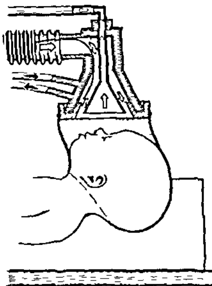


Fig 3 Face mask Outer double walled funnel with circulating water inner funnel and Plexiglas ring with plastic cap

rubber bag (1 l volume) filled to 1/3 of its capacity was interposed in the system (Fig 2)

The diluted expired air was led from the face mask to plastic bags by a pump (Reciprotor Type 406 g A/s Copenhagen Denmark) regulated by a transformer (Type E 401 AB 010-? 0 V Philips Holland) The volume of the air collecting system from the opening of the inner funnel to the collecting bags was 0 35 l The collecting bags were made of double laminated polyvinylchloride sheeting of 0 2 mm thickness Six bags could be applied simultaneously and connected one at a time with three way stopcocks

Equipment for environmental thermal control and temperature control (Figs 1 and 4)

The thermocontrolled chamber was made of double walled Plexiglas The inner dimensions were 55× 5×_0 cm and the temperature of the walls was controlled by circulating water To allow free access to the head of the infants and to hold the face mask in place the head end of the chamber had to be open but during examination it was carefully closed by a sheet of cloth The top of the chamber had two cylindrical openings closed by rubber gloves An insulating mattress of foam plastic was placed on the floor of the chamber

Room air (30 l/min) was supplied to the chamber by a fan The air was conducted through a humidifier of the same construction as used in the respiratory air supply system but with a maximum power of 100 W The temperature of the air was controlled by a heat exchanger (a copper cylinder of 30 cm length with six air channels of 6 mm diameter) From the heat-exchanger the air was led into the chamber through 12 holes in the foot end

Water supplying the walls of the chamber the walls

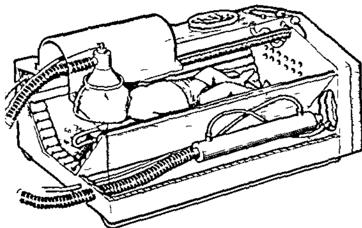


Fig 4 Thermocontrolled chamber with heat-exchanger

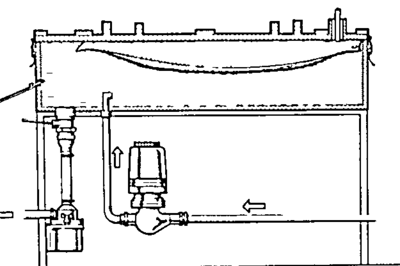


Fig 5 Apparatus for determination bag volume Plexiglas tank water volume meter exhausting pump and thermometer

f the face mask and the heat exchanger was circulated at a rate of 1 l/min from a water bath (Lauda Ultra thermostat Type CI 15/17 Messgeräte Werk Lauda Tauber Germany)

Temperatures was measured every minute by a six channel recorder (Strip chart recorder Type Z 8 Ellab instr Copenhagen Denmark) The six temperatures were Abdominal skin rectum (5 cm from anus) air within the face mask (between inner and outer funnel) air entering the chamber (foot end of the chamber) walls of the chamber (water leaving the walls) and water leaving the waterbath Another electric thermometer (Electric Universal thermometer Type TE 3 Ellab instr) was used to measure Rectal temperature at birth five additional skin temperatures and to perform hygrometric control of air in the chamber with a psychrometer (Type B 19 Ellab Instr) every 20 min

Equipment for determination of respiratory gas volume and composition

In order to avoid all changes in composition of collected gas during the measurement of gas volume a water replace method was used (Fig 5) The bags containing collected gas were enclosed in a plexiglass tank (100× 62×22 cm) subsequently filled with water of 24°C temperature through a volume meter (Type K Ae Aqua Metro Basel Switzerland) The gas volume was determined in duplicate and corrected to STPD taking hydrostatic pressure (Fig 6) water vapour pressure (50% re humidity) temperature and barometric pressure into account The fraction of oxygen and carbon dioxide in diluted expired air was measured with Nyons Diaferometer (Type MG Kipp & Zonen Delft Holland) The flow of air through the apparatus was 0 8 l/min The results of the analysis were recorded on a d c microammeter (Micrograf Type BD Kipp & Zonen) in conjunction with a channel selector (Type BAL Kipp & Zonen) One reading was done from each bag As reference gas for base line determination a bag containing gas from the tank delivering respiratory air was used All bags were examined within 8 hours after collection In order to assess the accuracy of the Diaferometer a measurement of a bag filled with calibration gas (21 467% O_2 and 1 073% CO_2) was performed after each series of bag analysis

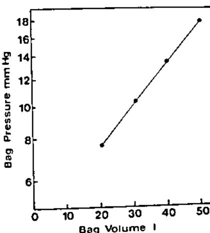


Fig 6 The relationship between hydrostatic pressure and bag volume at four different bag volumes

Calibration and Control of the Equipment

Determination of gas volume

The accuracy of the volume meter used to fill the water tank was checked by filling 17 bags with 20 00 l of water The measured mean volume was 20 00 l and S D 0 05 or 0 2% of the mean The error of the method was estimated from 30 randomly selected duplicate measurements and the results are given in Table I

Determination of gas composition

Calibration of the Diaferometer was performed according to the principles given by de Vos (4) For carbon dioxide calibration known volumes of pure CO_2 were mixed with 20 l CO_2 free air in bags A 60 ml glass syringe weighed and gas tight with a three way stopcock was used carefully avoiding all changes in temperature and pressure of the mixing gases The results from 20 experiments are given in Fig 7 As the response was found to be linear ($r=0$ 9996) a calibration factor at 23°C was estimated mean 0 0074 and S D 0 00016 or 2% of the mean ($n=20$) The error of the method was esti

Table 1 *The results of duplicate determinations*

Measurement	Mean value and range	Error of the method	Error of the method in % of mean
$\dot V_C$ l/min[b] (n=30)	6 09 5 39–6 35	0 017	0 3
$F_{IO_2}-F_{EO_2}$ vol% (n=34)	0 68 0 4–1 71	0 006	0 9
F_{ECO_2} F_{CO_2} vol% (n=34)	0 58 0 15–1 30	0 005	0 8
$\dot V_{O_2}$ ml/kg × min (n=?4)	7 0 4 64–11 63	0 4?	5 8
$\dot V_{CO_2}$ ml/kg × min (n= 4)	6 0? 3 79–9 78	0 47	7 8

Error of the method was estimated according the formula $= \sqrt{\frac{\Sigma d^2}{2n}}$

$\dot V$ Volume of diluted expired air collected in the bags per min

mated from 34 duplicate determinations and the results are given in Table 1

Two methods were used for oxygen calibration First ly technically pure oxygen (99 8% oxygen and 0 2% argon) was mixed in known volumes of CO_2 free air using the same technique as for CO_2 calibration Se ondly expired air was collected in bags and samples from these bags were analysed with repeated determina tion using the Haldane technique (8) Known volumes of expired air were subsequently diluted in CO_2 free air using the same technique as described for CO_2-calibra tion and analysed with the Diaferometer The calibra tion factor for oxygen at ?3°C in 19 experiments using pure oxygen mixed with air was mean 0 0098 and S D 0 000?5 or ? 5% of the mean In 13 experiments using expired air the factor was mean 0 0106 and S D 0 00032 or 3% of the mean There was no significant difference between the two calibration factors and a mean factor of 0 0100 was used The results of the calibration ex periments are given in Fig 8 The error of the method was estimated from 30 duplicate determinations and the results are given in Table 1

Bag control

In order to check the permeability of the bags calibra tion gas was kept in the bags in differing time periods Up to 8 hours no change in the fraction of oxygen and carbon dioxide could be detected

Environmental temperature control

The equilibration of temperatures within the chamber was slow and therefore the circulation of water had to be started 1 hour prior to any investigation During 2 hours (the duration of investigations) the temperatures of air coming into the chamber air in the face mask and wall temperature were stable (maximal change from mean 0 3°C) The difference between mean incoming air temperatures and mean wall temperatures in 41 in vestigations with a range in temperatures between ?8°C and 36°C was 0 17°C with a maximal difference of 0 5°C With no infants in the chamber no measurable differences in air temperature were found within the chamber

Humidity control

The relative humidity in the air of the chamber varied between 38% and 50% when infants were investigated at an environmental temperature of 34 C and between 45% and 58% in environmental temperature of ?9°C The relative humidity in the air of the face mask was esti mated to be approximately 50%

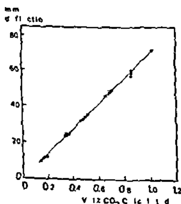


Fig 7 The results of the carbon dioxide calibration experiments The relationship between deflection on the recorder from the Nyons Diaferometer and volume % CO_2 calculated from gas mixture

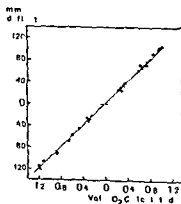


Fig 8 The results of the oxygen calibration experi ments The relationship between deflection on the re corder from the Nyons Diaferometer and volume % O_2 calculated from gas mixture with measured volumes of O_2 mixed with room air (positive values) and gas mix tures with measured volumes of expired air mixed with room air (negative values)

Air velocity

A meter for air velocity was used (Low velocity anemometer Type 55 D 80, Disa Elektronik A/S, DK 2739 Herlev, Denmark). The air velocity in the chamber did not exceed 5 cm/sec. In the face mask no measurements could be made but the mean air flow over the face of the infants was calculated to be less than 5 cm/sec.

Determination of V_{O_2} and V_{CO_2} in Infants

The total error of V_{O_2} and V_{CO_2} determination was estimated from 24 pairs of subsequent periods of gas collection in infants with the same degree of muscular activity. The results are given in Table I. The results of 12 examinations in infants in the basal state (activity zero) one to two hours after birth were V_{O_2} mean 5.17 ml/kg min (S.D. 0.36) and V_{CO_2} mean 4.39 ml/kg min (S.D. 0.39).

DISCUSSION

For the study of pulmonary gas exchange during the period immediately after birth an apparatus built on the open principle without respiratory valves was chosen. Diluted expired air was collected in bags over four minute periods and subsequently analysed for the bag volume and gas composition. A similar system has been reported by Karlberg (16). The advantages of this system are that the use of respiratory valves is avoided (see below) and that the measurements could start immediately after birth and continue at predetermined intervals without significant lag time. Continuous direct analysis of the expired air by Diaferometer was not done because of the long response time (more than 10 min) (17) and also because of the disturbing influence of temperature and pressure fluctuations in the delivery room on the sensitive Diaferometer.

Open systems with respiratory valves have been reported (7, 18, 22). The advantage with this system is that greater differences are achieved between inspiratory and expiratory oxygen and carbon dioxide concentrations. However, shortly after birth breathing is highly irregular and periods of crying are mixed with periods of rapid shallow breathing (5). The technical difficulties in constructing respiratory valves with accurate resistance and dead space were thus considered insurmountable.

In several investigations a closed system with a metabolic chamber has been used in studying newborn infants (6, 11, 12). However, in this system the infant has to stay in the closed chamber for 10–15 min to allow temperature equilibrium. Therefore this system cannot be used if measurements should start at birth.

Correct calibration of the gas analyser is essential to avoid systematic error in measurements. The easiest calibration procedure is to mix exact volumes of oxygen or carbon dioxide with known volumes of air. This procedure does not involve any problems for carbon dioxide but for oxygen it is difficult to get technically pure oxygen (4). In the present investigation calibration for oxygen was therefore also performed by measurements of diluted expired air. No significant difference was present between the two methods of calibration indicating that adding known volumes of oxygen to known volumes of air can be used for routine calibration of the analyser. The alcohol test has been used as reference both to open and closed systems (6, 16). This procedure is less suitable in the present method because incomplete combustion of alcohol will occure when air flow is less than approximately 30 l/min (according to results obtained in our laboratory).

In order to avoid rebreathing, 5–7 l of air/min passed through the face mask diluting expired air 6–10 times. If the error of V_{O_2} and V_{CO_2} determination was to be kept within 5% the accuracy of determination of the difference between F_{IO_2} and F_{EO_2} and F_{ECO_2} and F_{ECO_2} had to be 0.01 vol %. The results of the calibration experiments and duplicate determinations show that this degree of accuracy was obtained.

The gas analyser is built on the principle of thermoconduction of the gases and therefore not specific. Specific methods (as paramagnetic or electrochemical analysers for

oxygen and infrared analysers for carbon dioxide) would be more advantageous but the necessary high degree of sensitivity is difficult to obtain with these analysers (3) To use a mass spectrometer or a gas chromatograph are alternative solutions but they are not easily available and are costly

As the analyser used is not specific mixtures of other gaseous materials in the expired air could interfere with the determinations For instance a pilot study showed that if nitrous oxide was inhaled by the mother before delivery the pulmonary elimination of nitrous oxide by the infant after birth interfered with the analysis and made $\dot{V}_{O_2}$ and V_{CO_2} determinations impossible Thus only subjects who had not used nitrous oxide before birth could be investigated

The face mask used had the function of a small hood It was not in direct contact with the face of the infant The air flow through the face mask was 6 4 l/min and was the same as the maximal expiratory flow rate in crying newborn infants (106 ml/sec) (21) The risk of rebreathing was therefore minimal

The volume of the air collecting system between the face of the infant and the stop cocks of the bag was small (0 35 l) compared to the air flow of 6 4 l/min and thus the time lag for the determination was negligible (approximately 3–5 sec) in relation to the gas collection periods of 4 min

The great influence of environmental temperature on $\dot{V}_{O_2}$ in the newborn infants makes it necessary to do the measurements in thermocontrolled environment (for reviews see ref 2) In the present method circulating water was used to control air and wall temperature in the chamber The same principle has been used both in open and closed systems (1 12) The advantage of this principle over commercial incubators is that the difference between the air temperature and the wall temperature is minimal Thus the environmental temperature can be recorded as one temperature The temperature of the incoming air is regarded as representing the environmental air temperature in the present method This is a simplification as convectional heatloss from the infant does influence air temperature As has been pointed out by Gentz et al (6) it is impossible to take this influence into account in stating environmental air temperature

With the present method oxygen consumption in the basal condition in the first hours after birth was found to be 5 ml/kg min which is in good agreement with results reported earlier (1 10 13 20) The present method has been used in more than 50 investigations of newborn infants immediately after birth Some of the results have been published (24 27 28) Our experience has been that the apparatus described is well suited to these particular studies

REFERENCES

1 Bruck K Temperature regulation in the newborn infant *Biol Neonate 3* 65 1961

Bruck K Heat production and temperature regulation *In Physiology of the perinatal period* Meredith Corp New York 1970 Vol I p 493

3 Cunningham D A Montoye H J & Welch H G An evaluation of equipment for determining oxygen uptake *Res Quart 40* 851 1969

4 D Vos I C Metabolic rate determinations with the Diaferometer In *Shandon Instrument Applications* London 1967

5 Engstrom L Karlberg P Rooth G & Tunell R *The onset of respiration* A study of respiration and changes in blood gases and acid base balance Association for the Aid of Crippled Children New York 1966

6 Gentz J Persson B Hakkarainen J & Lindstedt S Apparatus measuring oxygen consumption and expired CO_2 and $\dot{V}CO_2$ in small subjects *J Appl Physiol 29* 731 1970

7 Golinko R J & Rudolph A M A valve for respiratory studies in infants *Pediatrics 27* 645 1961

8 Graham J & Haldane J S *Methods of air analysis* London 1934

9 Haselhorst G & Stromberger K Uber den Gasgehalt des Nabelschnurblutes vor und nach der Geburt des Kindes und uber den Gasaustausch in der Plazenta *Z Geburtsh Gynak 102* 16 1932

10 Hey E N The relation between environmental temperature and oxygen consumption in the new born baby *J Physiol 200* 589 1969

11 Hey E N Katz G & O Connell B The total

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THE INFLUENCE OF DIFFERENT ENVIRONMENTAL TEMPERATURES ON PULMONARY GAS EXCHANGE AND BLOOD GAS CHANGES AFTER BIRTH

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ABSTRACT Tunell R (Department of Paediatrics Karolinska Sjukhuset Stockholm Sweden) The influence of different environmental temperatures on pulmonary gas exchange and blood gas changes after birth Acta Paediatr Scand 64 57 1975 —The oxygen uptake (V_{O_2}) and respiratory exchange ratio (R) was determined during the first 20 min and at one and at 2 hours after birth in 16 healthy full term newborn infants studied in different environmental temperatures Arterial blood gases and acid–base balance were determined on repeated blood samples from the abdominal aorta The infants were grouped in a warm group ($n=10$) where efforts were made to avoid cooling after birth and a cold group ($n=6$) where a decrease in rectal temperature to a mean value of 35 4 C at 2 hours occurred Irrespective of environmental temperature V_{O_2} was approximately 10 ml/kg min during the first 8 min after birth thereafter decreasing to about 6–7 ml/kg min During the first 8 min the main increase in P_{aO_2} occurred and about 2 ml/kg min of the V_{O_2} was accounted for by changes in oxygen stores after birth At 16–20 min and at 60 min after birth a negative relationship was found between V_{O_2} and P_{aO_2} During the period 8–120 min after birth a close relationship was found between V_{O_2} and the degree of muscular activity Within 4–16 min after birth R values above 1 0 were regularly found simultaneously with the main decrease in P_{CO_2} In infants kept cold a tendency to hyperventilate was found probably elicited by cold stimuli The rapid drop in deep body temperature regularly seen after birth could thus not be explained by a limited ability to increase pulmonary gas exchange A high degree of evaporative heat loss a relatively low basal metabolic rate and a limited response in non shivering thermogenesis seem to be the main reasons for the heat loss after birth

KEY WORDS Newborn infants oxygen consumption carbon dioxide production environmental temperature blood gases

Immediately after birth the human newborn infant has a rapid decrease in skin temperature (10 11) and within the first hour after birth the rectal temperature decreases by about 2–3 C (2 7 10 28) The reason for this rapid cooling is mainly a high degree of evaporative heat loss from the relatively large skin surface area of the newborn infant (10 18) It has been suggested that the hypoxemia normally present at birth may lead to a depression in the metabolic response to cold and thus may contribute to the rapid decrease in body temperature (10 28) This statement has not been based on direct measurements of oxygen consumption and blood gases but on results achieved in studies of newborn infants a few days old breathing gas mixtures with low oxygen content (8 33) However this experimental situation differs in several respects from the situation at birth Furthermore Cross et al failed to confirm these reports (9)

In the present investigation a group of full term healthy newborn infants were stud

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thermal insulation of the new born baby *J Physiol 207* 683 1970
12 Hill J R The oxygen consumption of new born and adult mammals Its dependence on the oxygen tension in the inspired air and on the environmental temperature *J Physiol 149* 346 1959
13 Hill J R & Rahimtulla K A Heat balance and the metabolic rate of new born babies in relation to environmental temperature and the effect of age and of weight on basal metabolic rate *J Physiol 180* 239 1965
14 James L S Weisbrot I M Prince C E Holaday D A & Apgar V The acid–base status of human infants in relation to birth asphyxia and the onset of respiration *J Pediatr 52* 379 1958
15 James L S Acidosis of the newborn and its relation to birth asphyxia *Acta Paediatr Scand 49* Suppl *122* 17 1959
16 Karlberg P Determinations of standard energy metabolism in normal infants *Acta Paediatr Scand 41* Suppl *89* 1952
17 Karlberg P Moore R E & Oliver T K Jr Thermogenic and cardiovascular responses of the newborn baby to noradrenaline *Acta Paediatr Scand 54* 225 1965
18 Koch G Alveolar ventilation diffusing capacity and the A–a P_{O_2} difference in the newborn infant *Resp Physiol 4* 168 1968
19 Koch G & Wendel H Adjustment of arterial blood gases and acid base balance in the normal newborn infant during the first week of life *Biol Neonate 12* 136 1968
20 Levison H & Swyer P R Oxygen consumption and the thermal environment in newly born infants *Biol Neonate 7* 305 1964
21 Long E C & Hull W E Respiratory volume flow in the crying newborn infant *Pediatrics 27* 373 1961
22 Nelson N M Prodhom L S Cherry R B Lipsitz P J & Smith C A Pulmonary function in the newborn infant I *Pediatrics 30* 963 1962
23 Oliver T K Jr Demis J A & Bates G D Serial blood gas tensions and acid-base balance during the first hour of life in human infants *Acta Paediatr Scand 50* 346 1961
24 Persson B & Tunell R Influence of environmental temperature and acidosis on lipid mobilization in the human infant during the first two hours after birth *Acta Paediatr Scand 60* 385 1971
25 Pribylova H & Znamenacek K The effect of body temperature on the level of carbohydrate metabolites and oxygen consumption in the newborn *Pediatrics 37* 743 1966
26 Pribylova H & Novak L Influence of environmental temperature on the energetic and glyceride metabolism of pathological newborns during the first three hours of life *Biol Neonate 15* 315 1970
27 Tunell R The influence of different environmental temperatures on pulmonary gas exchange and blood gas changes after birth *Acta Paediatr Scand 63* 57 1974
28 Tunell R Copher D & Persson B The pulmonary gas exchange and blood gas changes in connection to birth In *Current concepts in neonatal intensive care* Warren H Green Inc St Louis USA In press
29 Wulf H Der Gasaustausch in der reifen Plazenta des Menschen *Z Geburtsh gynak 158* 117 1962

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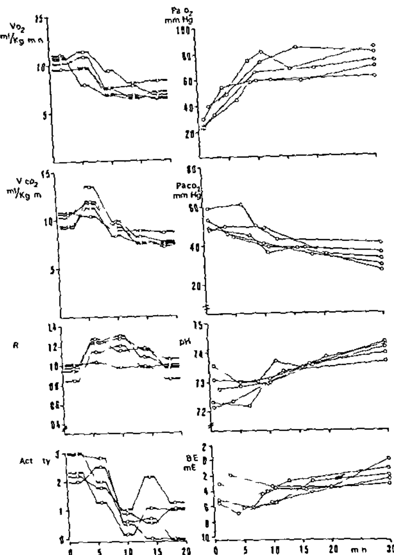


Fig 1 Sampling times and results of parameters measured *in five* warm infants

Case no	Parity	Duration of labour (hours)	Duration of 2nd stage (min)	Analgesia: Pud nerve block	Analgesia: Pethidin[a]	Mode of delivery presentation	Birth weight (g)	T (°C)
7	I	12	40	+	+	Vertex	4 255	34 8
1	III	7 5	5		+	Vertex	3 580	34 7
17	III	6	70		+	Occiput post	2 770	34 8
6	II	3	10			Vertex	2 960	33 2
8	IV	5	5			Vertex	3 170	33 5

Pethidin 50–100 mg administered 1–3 5 *hours* before delivery All infants had a similar postnatal course with an *initial* high degree of activity and pulmonary gas exchange followed by a rapid drop in activity and pulmonary gas exchange values Rapid *norma* ization of blood gases and acid–base balance was present in all infants

ied during the first 2 hours after birth at controlled environmental temperatures with serial determinations of pulmonary gas exchange and arterial blood gases and acid–base status Simultaneously the arterial blood concentrations of free fatty acids glycerol, glucose lactate and β hydroxy butyrate were determined The results of these biochemical measurements have been previously published (34)

The object of the present investigation was to determine the oxygen uptake during the period of hypoxemia immediately after birth and to see if differences in environmental temperature would influence the degree of pulmonary gas exchange and blood gas changes after birth

MATERIAL AND METHODS

The nature and the aim of the investigation was explained to the parents and all gave their consent

Sixteen healthy full term infants were investigated Clinical data on the infants and their mother are given in Figs 1 2 and 3

Procedure

At birth the infants were placed on an open infant bed consisting of a box (40×50 cm) with a 3 cm thick water filled mattress and a sterile towel When infants were to be studied in a warm environmental temperature the mattress and the towel were prewarmed to 38°C using an electric pad A heating lamp (250 W) was placed 70 cm above the mattress In investigations with cold environmental conditions the bed was kept at room temperature and no heating lamp was used All infants were carefully dried

After suction of the oro pharynx and as soon as spontaneous breathing had started a face mask was placed over the face of the infants and measurements of oxygen consumption $\dot{V}_{O_2}$ and carbon dioxide elimination $\dot{V}_{CO_2}$ were started using an apparatus described earlier (45) The measurements were performed continuously for 4 min periods during the first 20 min after birth and subsequently in two more periods of 4 min at one and at 2 hours after birth

As soon as possible after birth a cetheter was introduced into an umbilical artery and blood samples were withdrawn at predetermined intervals (for details see ref 34)

Within 5–10 min after birth the infants were transferred to a thermocontrolled chamber (45) The temperature of the chamber was set at four different levels 34 8 C (n=5) 33 5 C (n=5) 32 C (n=3) and 28 9 C (n=3) During the complete investigation period of 2 hours the infants were carefully watched and the degree of muscular activity assessed using a four point scale (45)

Temperature recordings

Within 2 min after birth the rectal temperature was measured using an electric thermometer (45) In the chamber rectal temperature and abdominal skin temperature were recorded every minute Five additional skin temperatures were measured every 0 min In order to get information about the mean skin temperature each skin temperature was weighted according to the surface area (in percent) that they represented (6) The skin temperatures were determined from (with weighting factor F) The xiphoid process (F 0 23) the abdominal skin in the mid line above the umbilical region (F 0 23) the lateral side of the left hand (F 0 05) the lateral side of the left upper leg (F 0 29) and the dorsal side of the left foot (F 0 05)

Arterial blood gas and acid base balance determinations

The blood samples (0 5 ml) were transferred to a heparinized and siliconiced glass syringe with a mercury lock The P_{O_2} determinations were performed with an open Clark electrode (19) The pH P_{CO_2} and acid–base determinations were performed by the Astrup micro equilibration technique (41) The blood gas determinations were made as soon as possible after sampling (always within 60 min) The results were corrected to the actual rectal temperature (40) All determinations were done in duplicate The error of P_{O_2} determination was 1 6 mmHg (n=185) and of pH determination 0 00 units (n=200)

RESULTS

Temperature

The 10 infants handled immediately in a warm environment and subsequently studied in an environmental temperature of 34 8°C and 33 5°C had rectal temperatures which in no infant fell below 36 7°C After an initial drop in skin temperature the temperature gradient between mean skin temperature and environmental temperature was 2 1–2 2°C (Table 1) As the changes in rectal temperature and skin temperature were similar in all 10 infants these infants were grouped together in group 1

The 6 infants immediately taken care of in room temperature and subsequently studied in an environmental temperature of 32°C and 28 9°C showed a heat loss with decreasing rectal temperatures and at 120 min all 6 infants had rectal temperatures below 36 5°C These 6 infants were grouped together in group 2 The 3 infants studied in an environmental temperature of 28 9°C

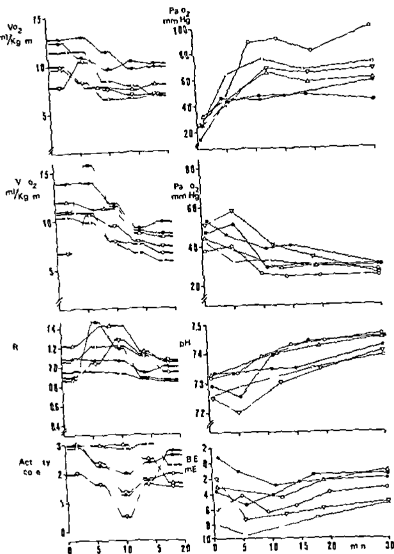


Fig 3 Sampling times and results of the parameters measured in cold infants

		Duration of		Analgesia					
Case no	Parity	labour (hours)	2nd stage (min)	Pud nerve block	Pethidin	Mode of delivery presentation	Birth weight (g)	*T* (°C)	Symbol
9	V	3	5			Vertex	3 370	32 0	○
13	II	5	10			Vertex	3 3[illegible]0	31 7	△
16	IV	4	10		+	Vertex	4 080	3[illegible] 0	●
18	I	7	0	+		Vertex	2 930	[illegible]8 8	□
0	III	4 5	10			Occiput post	2 660	[illegible]8 7	■
21	I	17	60	+	+	Vertex	3 030	29 8	▽

Pethidin 100 mg given more than 3 5 hours before delivery The pattern of changes in all parameters were the same as found in infants kept warm Infants with high $\dot{V}_{O_2}$ values 16–20 *min after birth* had low P_{O_2} values

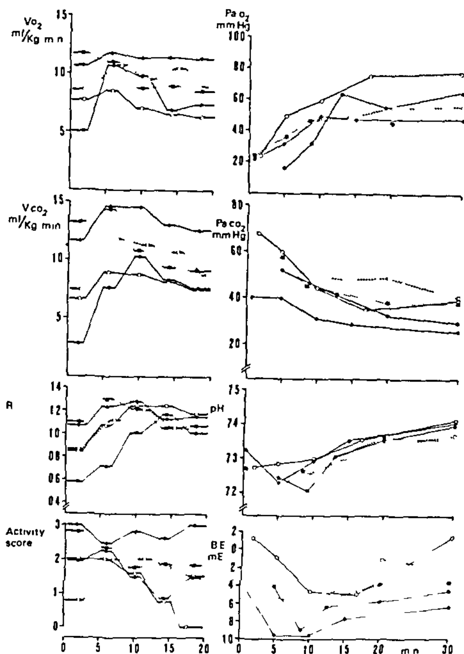


Fig 2 Sampling times and results of parameters measured in five warm infants

Case no	Parity	Duration of labour (hours)	Duration of 2nd stage (min)	Analgesia: Pud nerve block	Analgesia: Pethidin	Mode of delivery presentation	Birth weight (g)	*T* (°C)	Symbol
3	II					Ces sect [b]	3 050	34 6	○
19	III	3	5			Vertex	3 580	34 7	◆
23	I	7	50		+	Vertex	3 200	33 5	●
25	II	6	5			Vertex	3 820	33 4	□
29	III	3	5			Vertex	4 040	33 3	■

[a] Pethidin 75 mg given 2 hours before delivery
[b] Ces section because of narrow pelvis Spinal anaesthesia Case no 23 was clinically uneventful but during the first 4 min a low degree of pulmonary gas exchange was found The arterial P_{O_2} at 5 min of 17 mmHg indicates ineffective pulmonary gas exchange A persistently high degree of activity was found in cases 19 25 and 29 High $\dot{V}_{O_2}$ and low P_{O_2} values were found in these three cases Case 19 had a tendency to hyperventilate

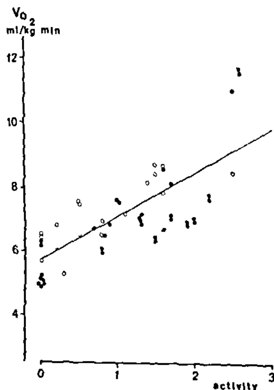


Fig 4 The relationship between V_{O_2} and the degree of muscular activity in the warm group within 8–174 min after birth ($r=0.72$ $n=94$ $p<0.001$) Infants studied at an environmental temperature of 33.5 C (O) and of 34.8°C (●)

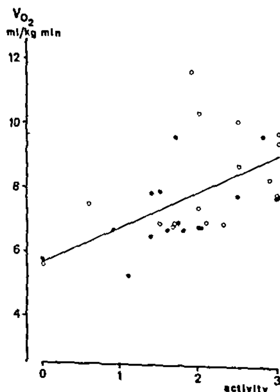


Fig 5 The relationship between V_{O_2} and the degree of muscular activity in the cold group within 8–174 min after birth ($r=0.56$ $n=44$ $p<0.001$) Infants studied in environmental temperature of 28.9°C (O) and of 32 C (●)

subsequent 40–50 min a decrease in the metabolic acidosis by about 4 mE occurred

The changes in pH reflected mainly the rapid changes in P_{aCO_2} and at 10 min after birth the infants of group 2 had significantly higher pH than infants of group 1 ($p<0.001$) At 2 hours 4 infants in group 2 had pH values above 7 45

DISCUSSION

The most obvious results in the present investigation were the high values of V_O (about twice the basal metabolic rate) and of V_{CO_2} (about three times the basal metabolic rate) found in the first 8 min after birth Furthermore differences in environmental temperature had no significant influence on V_{O_2} and V_{CO_2}

The initial high degree of pulmonary gas exchange is probably initiated by the same multiplicity of factors that are involved in the initiation of breathing after birth i e cold stimulation release of immersion acidic and hypercarbic stimulation of peripheral and central chemo sensing mechanisms (for review see ref 15) Other stimuli such as the perception of tactile and painful stimuli sound and gravity may contribute to an increased muscular activity and thus to a high metabolic rate During the subsequent adaptation to extra uterine life all these humoral and sensory stimuli are gradually decreasing with a concomitant decrease in metabolic rate However differences in the degree of spontaneous activity the influence of labor and delivery and drugs given to the mother during delivery modifies the response (Figs

Table 1. *Mean values and range in the temperature measurements in group 1 ("warm") and group 2 ("cold") infants*

Measurement	Group	Minutes after birth		
		2	40	120
Rectal temperature (°C)	I	38.0 37.8–38.2	37.2 36.9–37.5	37.3 36.7–37.9
	II	38.1 37.9–38.5	36.1 35.4–36.9	35.4 34.3–36.4
Rectal–skin temperature gradient (°C)	I		1.1 0.6–1.3	0.6 0.4–1.4
	II		1.6 0.3–2.1	1.3 1.1–1.6
Skin–environmental temperature gradient (°C)	I		2.1 1.3–3.0	2.2 1.6–2.9
	II		4.0 2.8–4.8	3.6 2.5–4.8

were, in contrast to the infants studied at 32°C, restless and very active during the examination period and meconium passage occurred. Infants in group 2 had a significantly greater skin–environmental temperature gradient than infants in group 1 (Table 1).

Pulmonary gas exchange and muscular activity

The results of the determinations of V_{O_2}, V_{CO_2}, the respiratory exchange ratio R (V_{CO_2}/V_{O_2}) and the degree of muscular activity are given in Table 2. The results from individual infants during the period 0–20 min after birth are given in Figs. 1, 2 and 3.

The pattern of changes in V_{O_2} and V_{CO_2} was similar in all infants irrespective of environmental temperature, and the highest level of pulmonary gas exchange was recorded within the first 8 min after birth. During the first 4 min period V_{O_2} regularly exceeded V_{CO_2}, but 4–16 min after birth R values above 1.0 were regularly found.

A close relationship was found between V_{O_2} and the degree of muscular activity during the period 8–124 min after birth. This relationship was unaffected by the difference in environmental temperature (Figs. 4 and 5).

Blood gas and acid base balance determinations

The results of P_{aO_2}, P_{aCO_2}, pH and base excess (BE) determinations are given in Table 3. The results from individual infants during the period 0–20 min after birth are given in Figs. 1, 2 and 3.

The pattern of changes in these parameters was similar in all infants irrespective of environmental temperature.

The main increase in P_{aO_2} took place 1–10 min after birth. After that period P_{aO_2} increased slowly but the values showed great variation. A close relationship was found between the values at 16–20 min after birth and the values at 60 and 120 min after birth respectively ($r=0.76$, $p<0.001$, $n=16$ and $r=0.79$, $p<0.001$, $n=15$). A negative relationship was found between V_{O_2} and P_{aO_2} at 16–20 min and at 60 min after birth ($r=0.63$, $p<0.01$, $n=16$ and $r=0.58$, $p<0.05$, $n=16$).

The main decrease in P_{aCO_2} occurred 5–15 min after birth. Infants studied in a heat losing environment had significantly lower P_{aCO_2} values at 10, 30, 60 and 120 min than infants in the "warm" group ($p<0.01$).

Initially (between 1–10 min) the degree of "metabolic" acidosis (given as BE) increased by approximately 3 mE. During the

64	116–119	120–124
±0 5	6 5±0 4	6 7±0 4
-10 9	5 2-8 6	5 0-8 4
±0 6	6 9±0 6	7 1±0 7
-10 3	5 3-9 [illegible]	5 1-9 4
±0 3	5 7±0 4	5 5±0 4
-7 8	3 9-7 7	4 3-8 0
±0 4	5 8±0 4	5 4±0 4
9-7 8	5 0-7 4	4 3-7 0
9±0 03	0 9±0 0-	0 9±0 02
8-1 1	0 7-0 9	0 8-1 0
9±0 04	0 9±0 0	0 8±0 03
7-1 0	0 8-0 9	0 7-0 8
±0 3	0 8±0 [illegible]	0 6±0 [illegible]
1- 5	0 0-1 5	0 0-1 7
7±0 1	1 3±0 5	1 5±0 5
[illegible]	0 0 3 0	0 0-3 0

0 (58–64)	1 0 (117–121)
74±4 5	82±5 0
54–100	47–105
$n=10$	$n=10$
4±7 2	74±5 0
51–95	53–90
$n=6$	$n=6$
38±1 7	34±0 9
25–44	27–36
$n=10$	$n=10$
8±1 2	29±1 5
5–3	3–34
=6	$n=6$
7 40±0 01	7 42±0 01
7 39–7 44	7 39–7 45
$n=9$	$n=10$
7 45±0 0	7 46±0 01
7 39–7 50	7 42–7 51
=6	$n=6$
−0 9±0 5	−1 1±0 6
+1 4–(−5 2)	+ 0–(−5)
$n=10$	$n=10$
−1 2±0 7	−1 ±0 7
+0 –(−3 6)	+1 1–(−3 2)
$n=6$	$n=6$

1 2 and 3) Therefore it is perhaps not surprising to find that a moderate change in one factor i e the degree of cold stimulation does not result in a significant change in the pattern and level of pulmonary gas exchange

The oxygen uptake in the first 8 min after birth was found to be approximately 10 ml/kg min and was in agreement with V_{O_2} values reported earlier (21) Simultaneously the main increase in arterial blood oxygen tension occurred The increase in blood oxygen content could be calculated to account for approximately 1 5–2 ml/kg min of V_{O_2} The remaining fraction of V_{O_2} (about 8 ml/kg min) most probably contributed to the cost of basal metabolic rate (approx 5 ml/kg min) and to the increased muscular activity mainly due to breathing (approx 3 ml/kg min) The present observation that the highest values of oxygen uptake were found within the first 8 min after birth simultaneously with the lowest values of arterial oxygen tension is evidence against the hypothesis that hypoxemia after birth will result in depressed oxygen consumption (10 28)

During the period 10–20 and 60 min after birth a negative relationship was found between the levels of V_{O_2} and P_{aO_2} In this time period the level of P_{aO_2} is mainly determined by the degree of the right to left shunt through the *ductus arteriosus* (31 32 36) A contributing factor to the low P_{aO_2} values found in infants with high V_{O_2} values could be an increased right to left shunt during periods of a high degree of activity with more or less obstructed expiration There were infants with low levels of P_{aO_2} both in the warm and the cold group and no significant difference in the P_{aO_2} values was present between the two groups (Table 3) In a study where one group of infants were exposed to a more pronounced cold stress than in the present investigation Stephenson et al (42) found lower values of P_{aO_2} at 20 and 60 min after birth in the cold group than in the warm group of infants The lower levels of P_{aO_2} found in

Table 2 *Mean value±S E M and range for V_{O_2} V_{CO_2} R and degree of muscular activity in group 1 (warm) and group 2 (cold) infants*

Measurement	Group	Minutes after birth 0–3	4–7	8–11	12–15	16–20	56–59
V_{O_2} ml/kg min	I *n*=10	9 6±0 6 5 1–11 7	10 2±0 4 8 0–11 8	8 5±0 5 7 0–11 3	8 0±0 5 6 4–11 5	7 8±0 5 6 2–11 3	7 5±0 5 6 1–11 6
	II *n*=6	10 7±0 8 7 9–12 6	10 2±0 8 7 8–11 3	8 4±0 7 6 5–11 2	8 1±0 6 6 6–10 1	8 0±0 6 6 7–10 0	7 8±0 8 6 4–11 6
V_{CO_2} ml/kg min	I *n*=10	9 2±0 9 2 9–13 2	11 6±0 7 7 7–14 5	10 0±0 6 7 7–14 5	8 8±0 6 6 5–13 0	8 0±0 6 6 3–12 6	7 1±0 6 5 2–11 8
	II *n*=6	10 8±0 9 6 8–13 9	12 1±0 9 9 8–15 6	9 9±0 8 7 6–11 8	8 1±0 4 6 7–9 0	7 7±0 6 5 8–9 8	6 8±0 5 5 8–8 7
R	I *n*=10	0 9±0 05 0 6–1 1	1 2±0 05 0 7–1 3	1 2±0 03 1 0–1 3	1 1±0 03 1 0–1 3	1 0±0 03 0 9–1 2	0 9±0 0[illegible] 0 8–1 1
	II *n*=6	1 0±0 05 0 9–1 2	1 2±0 09 1 0–1 5	1 2±0 06 0 9–1 5	1 0±0 06 0 9–1 1	1 0±0 04 0 8–1 1	0 9±0 0[illegible] 0 8–1 0
Activity	I *n*=10	2 3±0 2 0 8–3 0	2 2±0 2 1 3–2 8	1 3±0 3 0 2–2 8	1 3±0 3 0 0–2 6	1 1±0 3 0 0–3 0	1 5±0 3 0 4–2 6
	II *n*=6	2 7±0 2 2 0–3 0	2 5±0 2 1 6–3 0	1 9±0 4 0 6–3 0	2 3±0 3 1 7–3 0	2 3±0 2 1 6–2 7	1 7±0 1 1 4–2 0

Table 3 *Mean value±S E M and range for P_{aO_2} P_{aCO_2} pH and base excess (B E) in group 1 (warm) and group 2 (cold) infants*

Measurement	Group	Minutes after birth 1 (0 5–1 5)	5 (3 5–6 5)	10 (8 5–12)	17 (14–20)	30 (29–32)
P_{aO_2} mmHg	I	26±2 4 22–40 *n*=7	41±4 4 17–50 *n*=8	58±4 2 34–76 *n*=10	62±4 0 48–85 *n*=10	66±4 2 47–86 *n*=10
	II	26±2 4 16–34 *n*=6	49±4 3 40–65 *n*=5	64±8 4 42–87 *n*=5	61±6 0 47–82 *n*=6	66±7 9 43–100 *n*=6
P_{aCO_2} mmHg	I	54±3 2 41–67 *n*=7	52±2 7 40–61 *n*=8	44±1 7 32–50 *n*=10	37±1 8 28–48 *n*=10	35±1 6 26–40 *n*=10
	II	46±2 3 38–53 *n*=6	46±4 5 32–52 *n*=5	31±2 1 27–39 *n*=5	33±2 7 25–42 *n*=6	28±1 1 25–30 *n*=6
pH	I	7 30±0 02 7 22–7 37 *n*=8	7 26±0 01 7 22–7 31 *n*=8	7 29±0 01 7 22–7 32 *n*=10	7 35±0 01 7 30–7 38 *n*=10	7 40±0 01 7 36–7 44 *n*=9
	II	7 30±0 02 7 26–7 34 *n*=6	7 29±0 02 7 20–7 34 *n*=5	7 38±0 02 7 32–7 41 *n*=5	7 39±0 03 7 30–7 44 *n*=6	7 44±0 01 7 39–7 48 *n*=6
B E mE	I	−2 6±1 0 +1 2–(−5 6) *n*=7	−5 1±1 0 −1 0–(−9 5) *n*=8	−5 8±0 6 −3 2–(−9 5) *n*=10	−4 4±0 5 −2 7–(−7 5) *n*=10	−2 3±0 7 +1 4–(−5 7) *n*=10
	II	−3 6±1 3 +1 2–(−8 0) *n*=6	−5 5±1 5 −1 0–(−9 9) *n*=5	−5 2±0 9 −3 0–(−8 2) *n*=5	−3 9±1 0 −1 3–(−7 0) *n*=6	−2 9±0 7 −1 2–(−5 2) *n*=6

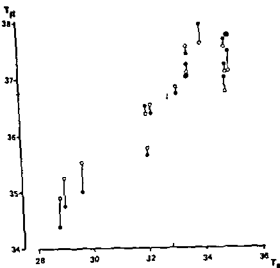


Fig 6 The relationship between rectal temperature T_R and environmental temperature T at 80 min (O) and at 1 0 min (●) after birth

edly shown that the newborn infant within the first hours after birth is able to increase its metabolic rate to a limited extent as a response to cold (3 16 18) However in these previous investigations the environ mental temperature was altered during the investigation period In the present study the environmental temperature was kept constant in each infant but there were dif ferences between the temperatures of the infants The thermogenic response to en vironmental temperature is primarily elicited by the temperature gradient between skin and environment (1) The small differences in skin environment temperature gradients between the warm and the cold group might have contributed to the lack of meta bolic response to cold exposure found in the present investigation

In conclusion the rapid drop in deep body temperature regularly seen after birth seems mainly to be caused by a high degree of evap orative heat loss (2 10 28) A relatively low basal metabolic rate and a limited response in non shivering thermogenesis are factors contributing to the heat loss The ability to increase pulmonary gas exchange does not seem to be a limiting factor An in creased metabolic rate associated with in creased muscular activity was found in in fants kept cold as well as in infants kept warm Due to increased heat loss by convection (17) the thermal effect to this type of metabolic rise is less than if non shivering thermogenesis had been the main reason

REFERENCES

1 Adamsons K Jr Gandy G M & James L S The influence of thermal factors upon oxygen con sumption of the newborn human infant *J Pediatr* 66 495 1965

2 Adamsons K Jr & Towell M E Thermal homeo stasis in the fetus and newborn *Anesthesiology* 26 531 1965

3 Berg D & Dorner J Das Verhalten des Saure-Basen-Haushalts am ersten Lebenstag unter beson derer Berucksichtigung der ersten Lebensminuten *Geburtsh Frauenheilkds* 29 980 1969

4 Beutnagel H Gauch D & Fabel H Blutgase und Sauren-Basen-Haushalt von normalen Neugebore nen am ersten Lebenstag *Z Geburtsh Gynak* 175 1 1971

5 — Der arterielle Sauerstoffpartialdruck beim Neuge borenen in den ersten Lebensminuten *Z Geburtsh Perinat* 176 117 1972

6 Bruck K Temperature regulation in the newborn infant *Biol Neonate* 3 65 1961

7 Burnhard E D & Cross K W Rectal tempera ture in the newborn infant after birth asphyxia *Brit Med J* II 1197 1958

8 Cross K W Tizard J P M & Trythall D A H The gaseous metabolism of the newborn infant breathing 15% oxygen *Acta Paediatr Scand* 47 217 1958

9 Cross K W Flynn D M & Hill J R Oxygen consumption in normal newborn infants during mod erate hypoxia in warm and cool environments *Pediatrics* 37 565 1966

10 Dahm L S & James L S Newborn temperature and calculated heat loss in the delivery room *Pedi atrics* 49 504 1972

11 Du J N H & Oliver T K Jr The baby in the delivery room *JAMA* 207 1507 1969

12 Engstrom L Karlberg P Rooth G & Tunell R *The onset of respiration* A study of respira tion and changes in blood gases and acid–base bal ance Association for Aid of Crippled Children New York 1966

13 Fischer W M & Toussaint W Über den Saure Basen Haushalt beim Neugeborenen Untersuchung en in den ersten Lebensminuten *Arch Gynaek* 199 187 1963

the present investigation were in agreement with the P_{aO_2} values reported earlier (3 4 5 12 24 32)

During the period 4–16 min after birth *R* values above 1 0 were regularly found In a group of infants with foetal distress even higher *R* values were found (44) During the period 1–10 min after birth an increase in the degree of metabolic acidosis was present which is in agreement with earlier studies (3 4 13 20 24 26 32 43) The simultaneous increase of metabolic acidosis and P_{aO_2} results in a release of CO_2 The decrease in P_{aCO_2} between 1 and 17 min after birth reflects a concomitant decrease in the total CO_2 stores The release of CO and the change in CO stores seem to be the main reasons for the increased postnatal CO_2 elimination A high ventilation/perfusion ratio would also result in an increased *R* value (35) However the arterial alveolar nitrogen gradient is high after birth indicating a low ventilation perfusion ratio (30) An elimination of nitrogen accumulated before birth could have influenced these results and a high ventilation/perfusion ratio might have contributed to the high *R* values found in the present investigation

Similar changes in *R* values as seen in the present investigation have been reported from studies of diving animals immediately after diving (25 37) The diving animal returning to the surface repays an oxygen dept with accumulated lactic acid in the musculature (37) The rapid increase in lactic acidosis seen in the newborn infant has been explained in the same way (38) Another explanation of the postnatal increase in lactic acidosis (and subsequent high *R* values) in infants after uneventful deliveries is a postnatal high degree of lactic acid production by the muscular tissues (26) The necessary intense muscular work mainly performed by the diaphragm (22 23) during a period of low P_{aO_2} and with low myoglobin content in the musculature (27) could thus result in a postnatal exercise oxygen dept similar to the oxygen dept during heavy exercise (29) It is not possible from the results in the present investigation to give a definite answer to the question about the origin of the postnatal acidosis

The low arterial blood P_{CO} values found in infants in a cold environment are in accordance with results reported earlier (14) The tendency to hyperventilation is probably partly elicited by cold stimulation

In the present investigation infants in group 1 were studied in an environmental temperature where cooling was avoided as much as possible but infants in group 2 were kept in a heat losing environment resulting in a decrease in rectal temperature similar to that found in infants subjected to routine care (12) No attempt was made to establish the thermo neutral zone in the immediate neonatal period This has already been done using apparatus better suited for this type of studies (6 16 18) In fact the infants of group 1 were kept in a heat gaining environment An environmental temperature of 32 5°C would have been required to obtain a rectal temperature between 36 5 and 37°C after 2 hours (Fig 6) This environmental temperature is equal to the reported critical temperature in full term infants during the first hours after birth (16 39)

In spite of the efforts to avoid cooling in group 1 an initial drop in skin and rectal temperature was present in all infants The transition from the intra uterine heatexchange over the placenta to the extra uterine heatexchange over the body surface makes this initial drop in skin and rectal temperature unavoidable (2)

Neither the relationship found between the degree of muscular activity and V_{O_2} nor the basal metabolic rate were altered by differences in environmental temperature This indicates a regulation of the basal metabolic rate during the first hours after birth independent of environmental temperature On the other hand, it has been repeat

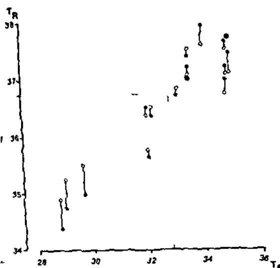


Fig 6 The relationship between rectal temperature T_R and environmental temperature T at 80 min (O) and at 1 0 min (●) after birth

edly shown that the newborn infant within the first hours after birth is able to increase its metabolic rate to a limited extent as a response to cold (3 16 18) However in these previous investigations the environmental temperature was altered during the investigation period In the present study the environmental temperature was kept constant in each infant but there were differencies between the temperatures of the infants The thermogenic response to environmental temperature is primarily elicited by the temperature gradient between skin and environment (1) The small differences in skin environment temperature gradients between the warm and the cold group might have contributed to the lack of metabolic response to cold exposure found in the present investigation

In conclusion the rapid drop in deep body temperature regularly seen after birth seems mainly to be caused by a high degree of evaporative heat loss (2 10 28) A relatively low basal metabolic rate and a limited response in non shivering thermogenesis are factors contributing to the heat loss The ability to increase pulmonary gas exchange does not seem to be a limiting factor An increased metabolic rate associated with increased muscular activity was found in infants kept cold as well as in infants kept warm Due to increased heat loss by convection (17) the thermal effect to this type of metabolic rise is less than if non shivering thermogenesis had been the main reason

REFERENCES

1 Adamsons K Jr Gandy G M & James L S The influence of thermal factors upon oxygen consumption of the newborn human infant *J Pediatr 66* 495 1965

2 Adamsons K Jr & Towell M E Thermal homeostasis in the fetus and newborn *Anesthesiology 26* 531 1965

3 Berg D & Dorner J Das Verhalten des Saure-Basen-Haushalts am ersten Lebenstag unter besonderer Berucksichtigung der ersten Lebensminuten *Geburtsh Frauenheilkds 29* 980 1969

4 Beutnagel H Gauch D & Fabel H Blutgase und Sauren-Basen-Haushalt von normalen Neugeborenen am ersten Lebenstag *Z Geburtsh Gynak 175* 1 1971

5 — Der arterielle Sauerstoffpartialdruck beim Neugeborenen in den ersten Lebensminuten *Z Geburtsh Perinat 176* 117 1972

6 Bruck K Temperature regulation in the newborn infant *Biol Neonate 3* 65 1961

7 Burnhard E D & Cross K W Rectal temperature in the newborn infant after birth asphyxia *Brit Med J II* 1197 1958

8 Cross K W Tizard J P M & Trythall D A H The gaseous metabolism of the newborn infant breathing 15% oxygen *Acta Paediatr Scand 47* 217 1958

9 Cross K W Flynn D M & Hill J R Oxygen consumption in normal newborn infants during moderate hypoxia in warm and cool environments *Pediatrics 37* 565 1966

10 Dahm L S & James L S Newborn temperature and calculated heat loss in the delivery room *Pediatrics 49* 504 197

11 Du J N H & Oliver T K Jr The baby in the delivery room *JAMA 207* 1502 1969

12 Engström L Karlberg P Rooth G & Tunell R *The onset of respiration* A study of respiration and changes in blood gases and acid–base balance Association for Aid of Crippled Children New York 1966

13 Fischer W M & Toussaint W Über den Saure Basen Haushalt beim Neugeborenen Untersuchungen in den ersten Lebensminuten *Arch Gynaek 199* 187 1963

14 Gandy G M Adamsons K Jr Cunningham N Silverman W A & James L S Thermal environment and acid-base homeostasis in human infants during the first few hours of life *J Clin Invest 43* 751 1964
15 Hamed H S Jr Respiration and the respiratory system *In Physiology of the perinatal period Meredith Co New York 1970 Vol 1 p 43*
16 Hey E N The relation between environmental temperature and oxygen consumption in the new born baby *J Physiol 200* 589 1969
17 Hey E N Katz G & O Connell B The total thermal insulation of the new born infant *J Physiol 207* 638 1970
18 *Hill J R & Rahimtulla K A Heat balance and* the metabolic rate of new born babies in relation to environmental temperature and the effect of age and of weight on basal metabolic rate *J Physiol 180* 239 1965
19 Holmgren A A simple cuvette for measurement of P_{O_2} in minute amounts of blood *Scand J Clin Lab Invest 17* 195 1965
20 James L S Weisbrot I M Prince C L Holiday D A & Apgar V The acid-base status *of human infants in relation to birth asphyxia and* the onset of respiration *J Pediatr 52* 379 1958
21 Karlberg P Determinations of standard energy metabolism in normal infants *Acta Paediatr Scand 41* Suppl *89* 1952
22 Karlberg P Cherry R B Escardó F E & Koch G Respiratory studies in newborn infants *Acta Paediatr Scand 51* 121 1962
23 Karlberg P & Koch G Respiratory studies in newborn infants *Acta Paediatr Scand 135* 121 1962
24 Koch G & Wendel H Adjustment of arterial blood gases and acid base balance in the normal newborn infant during the first week of life *Biol Neonate 12* 136 1968
25 Kooyman G L Kerem D H Campbell W B & Wright J J Pulmonary gas exchange in freely diving wedell seals *Resp Physiol 17* 283 1973
26 Künzel W Dening H H Caffier H & Wulf H Der Säure Basen Status in der Neugeborenenperiode *Z Geburtsh Gynaek 170* 231 1969
27 Longo L D & Koos B J Myoglobin in the foetal muscle and its role in oxygenation In *Foetal and neonatal physiol* Proceedings of the Sir Joseph Barcroft centenary symposium Cambridge University Press *1973 p 292*
28 Miller D L & Oliver T K Jr Body temperature in the immediate neonatal period The effect of reducing thermal losses *Am J Obstet Gynec 94* 964 1966
29 Naimark A Wasserman K & McIlroy M B Continuous measurement of ventilatory exchange ratio during exercise *J Appl Physiol 19* 644 1964
30 Nourse C H & Nelson N M Uniformity of ventilation in the newborn infant Direct assessment of the arterial alveolar N_2 difference *Pediatrics 43* 226 1969
31 Oh W Arcilla R A Lind J & Gessner I H Arterial blood gas and acid base balance in the new born infant Effect of cord clamping at birth *Acta paediatr Scand 55* 593 1966
32 Oliver T K Jr Demis J A & Bates G D Serial blood gas tensions and acid-base balance during the first hour of life in human infants *Acta Paediatr Scand 50* 346 1961
33 Oliver T K Jr & Karlberg P Gaseous metabolism in newly born human infants *Am J Dis Child 105* 427 1963
34 Persson B & Tunell R Influence of environmental temperature and acidosis on lipid mobilization in the human infant during the first two hours after birth *Acta Paediatr Scand 60 385 1971*
35 Rahn H & Farhi L E Ventilation perfusion and gas exchange—the $\dot{V}_A/\dot{Q}$ concept In (eds) *W O Fenn & H Rahn Handbook of Physiology* Section 3 *Respiration* Vol I Washington D C American Physiological Society 1965 p 735
36 Saling E Neue Untersuchungsergebnisse über den Kreislauf des Kindes unmittelbar nach der Geburt *Arch Gynaek 194* 287 1960
37 Scholander P F Experimental investigations on *the respiratory function in diving mammals and* birds *Hvalradets Skrifter* Norske Videnskap Akad Oslo 22 1940
38 — Experimental studies on asphyxia in animals *In Oxygen supply to the human foetus* Blackwell Oxford 1959 p 267
39 Scopes J W Metabolic rate and temperature control in the human baby *Br Med Bull 22* 88 1966
40 Severinghaus J W Blood gas concentrations In *Handbook of physiology* section 3 *Respiration* Washington D C American Physiological Society 1965 Vol 2 p 1475
41 Siggaard Andersen O Engel K Jörgensen K & Astrup P A micromethod for determination of pH carbon dioxide tension base excess and standard bicarbonate in capillary blood *Scand J Clin Lab Invest 12* 172 1960
42 Stephenson J M Du J N & Oliver T K Jr *The effect of cooling on blood gas tensions in new* born infants *J Pediatr 76* 848 1970
43 Thalme B Electrolyte and acid-base balance in fetal and maternal blood *Acta Obstet Gynec Scand 45* Suppl 8 1967
44 Tunell R Copher D & Persson B The pulmonary gas exchange and *blood gas changes in connection to birth In Current concepts in neonatal intensive care* Warren H Green Inc St Louis In press 1974
45 Tunell R Method for determination of pulmonary gas exchange in connection to birth *Acta Paediatr Scand 63* 49 1974

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PULMONARY MECHANICS IN INFANTS SURVIVING SEVERE NEONATAL RESPIRATORY INSUFFICIENCY

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ABSTRACT Ahlstrom H (Departments of Paediatrics and Clinical Physiology University Hospital Lund Sweden) Pulmonary mechanics in infants surviving severe neonatal respiratory insufficiency Acta Paediatr Scand 64 69 1975.—Pulmonary mechanics was studied in 24 survivors of severe neonatal ventilatory insufficiency 15 infants had idiopathic respiratory distress syndrome (IRDS) 6 recurrent severe apnoeic spells and 3 postasphyxia syndrome Of the infants with IRDS 5 were treated with intermittent positive pressure ventilation (IPPV) 3 with continuous positive airway pressure (CPAP) via an endotracheal tube and 7 with CPAP applied via a face chamber The other infants were all treated with IPPV IPPV treated infants generally had lower than expected values of dynamic compliance and pulmonary conductance particularly after prolonged treatment All infants treated with CPAP via a face chamber had normal mechanics but a trend towards obstruction of the airways after varying periods of time was observed in most infants irrespective of diagnosis or treatment One infant treated with CPAP via an endotracheal tube and given pure oxygen for a long time had gross abnormalities suggesting bronchopulmonary dysplasia Measurement of pulmonary conductance appears to be a reliable prognostic tool as concerns pulmonary symptoms later in infancy

KEY WORDS Pulmonary mechanics intermittent positive pressure ventilation continuous positive airway pressure infants face chamber

During the last few years intermittent positive pressure ventilation (IPPV) has become widely used in the treatment of various forms of severe respiratory insufficiency in the neonatal period A higher survival rate has been followed by an increasing number of infants with respiratory symptoms persisting long after the cessation of the IPPV These symptoms might be related to the primary disease or be caused by high oxygen concentrations or the hazards of IPPV i e damage from the indwelling tracheal tube and high inflation pressure With the introduction of continuous positive airway pressure (CPAP) without intubation in the treatment of severe IRDS (1 2 18) it might be possible to analyse to what extent persisting symptoms are due to the hazards specific for IPPV When CPAP with a face chamber was introduced a randomized study was planned comparing CPAP treatment with IPPV both as regards immediate results and residual changes in pulmonary function (1) For ethical reasons this study could not be carried out since it was obvious that early treatment with CPAP was superior

Several authors have described the microscopic picture of the lungs of infants dying during IPPV or of lung biopsies from survivors (8 10 17 28) Since only few studies of pulmonary mechanics after mechanical

14 Gandy G M Adamsons K Jr Cunningham N *Silverman W A & James L S* Thermal environment and acid-base homeostasis in human infants during the first few hours of life *J Clin Invest 43* 751 1964
15 Hamed H S Jr Respiration and the respiratory system In *Physiology of the perinatal period* Meredith Co New York 1970 Vol I p 43
16 Hey E N The relation between environmental temperature and oxygen consumption in the new born baby *J Physiol 200* 589 1969
17 Hey E N Katz G & O Connell B *The total* thermal insulation of the new born infant *J Physiol 207* 638 1970
18 Hill J R & Rahimtulla K A Heat balance and the metabolic rate of new born babies in relation to environmental temperature and the effect of age and of weight on basal metabolic rate *J Physiol 180* 239 1965
19 Holmgren A A simple cuvette for measurement of P_{O_2} in minute amounts of blood *Scand J Clin Lab Invest 17* 195 1965
20 James L S Weisbrot I M Prince C E Holaday D A & Apgar V The acid-base status of human infants in relation to birth asphyxia and the onset of respiration *J Pediatr 52* 379 1958
21 Karlberg P Determinations of standard energy metabolism in normal infants *Acta Paediatr Scand 41* Suppl *89* 1952
22 Karlberg P Cherry R B Escardó F E & Koch G Respiratory studies in newborn infants *Acta Paediatr Scand 51* 121 1962
23 Karlberg P & Koch G Respiratory studies in newborn infants *Acta Paediatr Scand 135* 121 1962
24 Koch G & Wendel H Adjustment of arterial blood gases and acid base balance in the normal newborn infant during the first week of life *Biol Neonate 12* 136 1968
25 *Kooyman G L Kerem D H Campbell W B &* Wright J J Pulmonary gas exchange in freely diving wedell seals *Resp Physiol 17* 283 1973
26 Kunzel W Dening H H Caffier H & Wulf H Der Saure Basen Status in der Neugeborenenperiode *Z Geburtsh Gynaek 170* 231 1969
27 Longo L D & Koo B J Myoglobin in the foetal muscle *and its role in oxygenation In Foetal and neonatal physiol* Proceedings of the Sir Joseph Barcroft centenary symposium Cambridge University Press 1973 p 292
28 Miller D L & Oliver T K Jr Body temperature in the immediate neonatal period The effect of reducing thermal losses *Am J Obstet Gynec 94* 964 1966
29 Naimark A Wasserman K & McIlroy M B Continuous measurement of ventilatory exchange ratio during exercise *J Appl Physiol 19* 644 1964
30 Nourse C H & Nelson N M Uniformity of ventilation in the newborn infant Direct assessment of the arterial *alveolar N_2 difference Pediatrics 43* 226 1969
31 Oh W Arcilla R A Lind J & Gessner I H Arterial blood gas and acid base balance in the new born infant Effect of cord clamping at birth *Acta paediatr Scand 55* 593 1966
32 Oliver T K Jr *Demis J A* & Bates G D Serial blood gas tensions and acid-base balance during the first hour of life in human infants *Acta Paediatr Scand 50* 346 1961
33 Oliver T K Jr & Karlberg P Gaseous metabolism in newly born human infants *Am J Dis Child 105* 427 1963
34 *Persson B & Tunell R Influence* of environmental temperature and acidosis on lipid mobilization in the human infant during the first two hours after birth *Acta Paediatr Scand 60* 385 1971
35 Rahn H & Farhi L E Ventilation perfusion and gas exchange—the V_A/Q concept In (eds) *W O Fenn & H Rahn Handbook of Physiology* Section *3 Respiration* Vol *I* Washington D C American Physiological Society 1965 p 735
36 Saling E Neue Untersuchungsergebnisse uber den Kreislauf des Kindes unmittelbar nach der Geburt *Arch Gynaek 194* 287 1960
37 Scholander P F Experimental investigations on the respiratory function in diving mammals and birds *Hvalrådets Skrifter* Norske Videnskaps Akad Oslo 22 1940
38 — Experimental studies on asphyxia in animals In *Oxygen supply to the human foetus* Blackwell Oxford 1959 p 267
39 Scopes J W Metabolic rate and temperature control in the human baby *Br Med Bull* 22 88 1965
40 *Severinghaus J W Blood* gas *concentrations* In *Handbook of physiology* section 3 *Respiration* Washington D C American Physiological Society 1965 Vol 2 p 1475
41 Siggaard Andersen O Engel K Jorgensen *K &* Astrup P A micromethod for determination of pH carbon dioxide tension base excess and stand*ard bicarbonate in capillary blood Scand J Clin Lab Invest 12* 172 1960
42 Stephenson J M Du J N & Oliver T K Jr The effect of cooling on blood gas tensions in new born infants *J Pediatr 76* 848 1970
43 Thalme B Electrolyte and acid base balance in fetal and maternal blood *Acta Obstet Gynec Scand 45* Suppl 8 1967
44 Tunell R Copher D & Persson B The pulmonary gas exchange and blood gas changes in connection to birth In *Current concepts in neonatal intensive care* Warren H *Green Inc St* Louis In press 1974
45 Tunell R Method for determination of pulmonary gas exchange in connection to birth *Acta Paediatr Scand 63* 49 1974

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Oxygen conc			
100-81%	80-61%	60-41%	40-21%
Duration hours			
–	27	167	274
–	54	45	36
–	27	40	–
–	79	45	–
–	–	36	104
–	–	–	117
–	–	–	384
–	7	–	67
–	–	–	199
–	–	–	232
–	–	–	146
–	9	–	37
–	37	–	–
–	3	6	–
–	–	–	16
–	–	–	36
33	–	15	–
–	–	–	6
–	–	52	44
–	–	–	36
–	–	–	5
–	–	18	–
–	–	–	70
–	–	–	88

resistance The reciprocal of Rf(1) functional pulmonary *conductance* (*Gf*(1)) was calculated from the mean value of repeated Rf(1) measurements The pressure and flow were also displayed on a direct writing recorder (Mingograph 81 Siemens Elema) The calculations were based on several measurements each comprised of eight consecutive breaths Information on the breathing pattern was obtained after the examination when the computer drew diagrams of e g flow versus pressure and volume versus pressure The investigation was made on non sedated sleeping infants at rest and during carbon dioxide induced hyperventilation All data of Cdyn(1) and Gf(1) presented were obtained during hyperventilation The method and procedure have been described in detail elsewhere (3 4)

All 74 surviving infants treated with IPPV or CPAP because of severe respiratory insufficiency in the Neonatal Units in Lund and Malmö during 18 months were examined Data and diagnoses of the infants appear in Table 1

Fifteen infants with *idiopathic respiratory distress syndrome* (IRDS) were studied The diagnostic criteria for IPPV or CPAP treatment were grunting retractions tachypnoea and an arterial oxygen tension below 70 mmHg after breathing 100% oxygen for 10 minutes (hyperoxia test) A pulmonary X ray showing a reticulogranular picture was considered to support the diagnosis The symptoms developed within the first day of life Five of the infants with IRDS were treated with IPPV In 2 of the patients (nos ? 4) IPPV was started urgently because of apnoeic spells and severe cyanosis no hyperoxia test was performed just *before treatment* Ten patients with IRDS were treated with CPAP 3 via an endotracheal tube according to Gregory et al (18) and 7 via a face chamber (1 2) Since very good preliminary results were obtained after treatment with the face chamber (1) no more infants with IRDS were treated with IPPV or CPAP with intubation

In 6 infants the diagnosis of recurrent apnoea in the prematurity (41) here called *respiratory insufficiency syndrome* (RIS) was established They had no signs of respiratory failure during the first day of life After a varying time they all got apnoeic spells with bradycardia whereupon IPPV was started No grunting was observed

Three infants were mechanically ventilated because of *postasphyxia syndrome* (31) They had all shown signs of intra-uterine asphyxia Respiratory insufficiency developed soon after birth with increasing hypoxia acidosis and apnoeic spells IPPV was started if oxygen tension was below 70 mmHg at hyperoxia test or after repeated apnoeic spells with bradycardia Pulmonary X ray showed in these cases signs of atelectases and cardiomegaly

The ventilators used were Loosco Amsterdam Infant Ventilator (24) and Servoventilator 900 (Siemens-Elema) (27) During the treatment the infants were lying in an intensive care crib (14) allowing frequent (every 2 hours) changes between the left and right lateral position for drainage of the lungs All infants artificially ventilated or treated with CPAP via an endotracheal tube were intubated through the nose with a Portex endotracheal tube (Nos 2 5-3 5) Suction after instillation of 0 3-0 5 ml of saline was performed at least hourly Reintubation was performed after 4 days or more often on signs of occlusion of the tube

All infants were treated according to wellknown principles including temperature and pulse control glucose infusion correction of acidosis with bicarbonate and antibiotics if signs of infection appeared The pressure and the oxygen concentration during IPPV and CPAP breathing were adjusted to give a normal arterial pH P_{CO_2} and Base Excess and an arterial oxygen tension of 50-90 mmHg The peak pressure never exceeded 40 cm H_2O during IPPV and during CPAP treatment the infants were breathing against at most 10 cm H_2O (Table 1)

The first investigation of pulmonary mechanics was performed after the period of intensive care and in most cases when the infants were well enough to be sent home from the hospital All except 3 infants were re-examined at varying ages during the first year of life (Tables 2 and 3)

The findings were compared with a material of normal subjects (4) Body height was found a suitable measure in correction for body size as concerns pulmonary mechanics properties in normal infants (4) A

Table I *Data at birth diagnosis and treatment data of the infants*

No	Sex	Birth (weight)	Gestational age (weeks)	Diagnosis	Treatment	Age of start (hours)	Duration (hours)	Peak inflation pressure (cm H_2O)	CPAP (cm H_2O)
1	M	1 900	31	Preterm AGA[a] IRDS	IPPV	20	463	20–30	
2	F	1 280	32	Preterm AGA IRDS	IPPV	10	135	20–40	
3	F	1 920	33	Preterm AGA IRDS	IPPV	7	67	20–30	
4	F	2 150	36	Preterm AGA IRDS	IPPV	11	126	20–30	
5	F	1 220	28	Preterm AGA IRDS	IPPV	96	140	20–30	
6	M	1 230	33	Preterm AGA RIS	IPPV	80	117	20–30	
7	M	1 330	29	Preterm AGA RIS	IPPV	120	384	20–30	
8	F	2 140	34	Preterm AGA RIS	IPPV	120	67	20–30	
9	F	1 260	31	Preterm AGA RIS	IPPV	36	199	20–30	
10	F	1 330	28	Preterm AGA RIS	IPPV	30	232	20–30	
11	F	1 560	31	Preterm AGA RIS	IPPV	31	146	20–30	
12	M	3 810	40	Postasphyxia syndrome	IPPV	8	46	30–40	
13	M	3 560	42	Postasphyxia syndrome	IPPV	10	37	20–30	
14	M	2 000	31	Preterm AGA postasphyxia syndr	IPPV	5	9	30	
15	M	2 550	34	Preterm AGA IRDS	CPAP tube	8	16		10
16	M	2 570	38	Preterm AGA IRDS	CPAP tube	6	36		10
17	F	2 300	36	Preterm AGA IRDS	CPAP tube	72	48		10
18	F	2 250	35	Preterm AGA IRDS	CPAP f c[b]	5	7		10
19	F	2 330	34	Preterm AGA IRDS hypothyreos	CPAP f c	24	96		4–8
20	M	2 500	35	Preterm AGA IRDS	CPAP f c	11	36		5
21	M	1 830	35	Preterm AGA IRDS	CPAP f c	5	5		10
22	M	3 130	35	Preterm AGA IRDS	CPAP f c	8	18		8
23	M	2 420	38	Preterm AGA IRDS Duplex II	CPAP f c	10	20		4
24	M	1 850	32	Preterm AGA IRDS	CPAP f c	20	88		6

[a] AGA appropriate for gestational age
[b] f c face chamber

ventilation or CPAP treatment have been made (7, 9 11 37) the present survey was performed in order to examine the extent of remaining manifest or subclinical abnormalities of lung function It was realized from the beginning that it would probably not be possible to establish cause effect relationships since abnormalities could be due to the pathological condition *per se* or the treatment

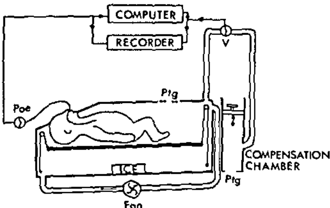


Fig 1 Schematic drawing of the equipment *Ptg* indicates the pneumotachograph screens The infant lies on a board which can be moved up and down *Poe* and *V* indicate transducers for oesophageal pressure and air flow measurement

METHODS AND MATERIAL

Oesophageal pressure was measured via a fluid filled catheter inserted through the nose to the middle third of the oesophagus and connected to a low-compliant pressure transducer (EMT 34 Siemens Elema) Lung volume changes were measured with a volume displacement body plethysmograph (Fig 1) Great care was taken to avoid undue phase shift between the flow and pressure signals since this may cause considerable errors in the measurements of dynamic compliance and resistance (3) The signals for pressure and flow were fed on line to a computer (PDP 8 I Digital Equipment) for calculation of tidal volume (V_T) breathing frequency (*f*) minute ventilation ($\dot{V}_E$) dynamic compliance Cdyn(1)) functional pulmonary resistance (Rf(1)) and work rate against pulmonary

Table 2 *Measured data on 14 infants treated with mechanical ventilation*

Case no	Age (weeks)	Height (cm)	f_{res} (min^{-1})	V_T (ml)	$\dot{V}_{IE}$ (l/min)	f_h (min^{-1})	V_T range (ml)	$\dot{V}_{IE}$ range (l/min)	$Cdyn(l)_h$ (ml/cm H_2O)	$Gf(l)_h$ [(l/s)/cm H_2O]
1	14	54	54	19 3	1 0	70	17 2–26 9	0 9–1 4	2 2	0 013
	21	59	50	11 1	0 6	55	8 6–20 1	0 5–1 1	1 9	0 012
	30	63	52	33 2	1 6	57	33 8–45 1	1 8–2 6	5 1	0 026
	47	70	46	40 0	1 8	51	44 3–64 9	2 1–3 0	4 5	0 025
2	20	55	44	28 5	1 2	46	13 4–57 0	1 2–2 6	5 5	0 016
	53	69	36	65 4	2 4	37	49 4–103	1 9–3 8	8 8	0 017
3	7	46	63	19 2	1 2	82	10 8–40 6	0 8–3 3	4 4	0 045
4	6	50	43	17 5	0 8	45	12 1–26 9	0 7–1 2	3 9	0 009
	23	62	28	55 4	1 3	31	42 1–95 2	1 2–3 0	12 8	0 027
5	10	47	41	18 9	0 9	40	20 4–30 4	0 8–1 2	4 4	0 011
	29	64	40	38 4	1 4	53	25 3–86 7	1 3–3 1	13 8	0 036
6	13	49	51	19 7	1 0	58	10 0–40 3	0 9–1 6	5 2	0 018
	52	70	22	69 0	1 6	29	68 3–97 3	1 6–2 5	13 6	0 022
7	10	51	94	12 6	1 2	80	9 4–32 5	0 8–2 1	2 4	0 013
	49	64	41	45 9	1 9	45	41 6–64 0	1 7–3 0	7 3	0 022
8	6	45	44	24 3	1 0	45	16 7–34 3	0 9–1 5	5 9	0 016
9	13	47	49	17 1	0 8	57	13 3–22 8	0 5–1 3	1 7	0 006
	19	54	68	13 1	1 1	43	10 7–34 7	0 7–1 5	3 8	0 011
	40	65	42	61 9	2 6	47	43 7–86 1	1 8–3 3	14 4	0 029
10	10	45	53	19 6	1 0	55	11 3–20 1	0 9–1 1	2 2	0 008
	21	56	59	23 7	1 4	58	21 8–44 7	1 0–2 3	4 0	0 016
11	6	44	35	22 1	0 8	43	22 2–29 3	0 9–1 1	6 9	0 031
12	2	51	64	16 2	1 0	95	17 9–25 3	0 8–2 1	3 5	0 029
	35	[illegible]	30	69 8	2 1	37	52 4–104	1 8–3 8	11 9	0 018
13	5	54	45	30 9	1 4	41	27 0–57 0	1 0–2 4	7 2	0 019
	32	71	33	44 3	1 7	34	61 3–128	1 7–4 5	9 3	0 033
14	6	46	54	17 8	1 0	59	17 0–32 3	0 8–1 9	4 5	0 015

Obtained at highest $\dot{V}_{IE}$

Table 3 *Measured data on 10 infants treated with CPAP*

Case no	Age (weeks)	Height (cm)	f_{res} (min^{-1})	V_T (ml)	$\dot{V}_{IE}$ (l/min)	f_h (min^{-1})	V_T range (ml)	$\dot{V}_{IE}$ range (l/min)	Cdyn $(l)_h$ (ml/cm HO)	$Gf(l)_h$ [(l/s)/cm H_2O]
15	3	45	65	12 7	0 8	69	6 2–20 8	0 6–2 0	3 7	0 019
16	3	46	55	16 8	1 4	59	13 0–23 1	0 7–1 5	3 0	0 018
	10	54	40	28 6	1 2	45	27 0–54 0	1 1–2 6	6 5	0 024
17	14	55	66	25 9	1 7	5	22 8–30 2	1 1–2 0	2 9	0 005
	25	64	53	42 0	2 2	47	38 7–62 3	2 0–3 0	4.5	0 016
18	4	45	70	29 7	2 0	63	34 2–45 3	1 8–2 6	7 5	0 036
	42	71	42	60 5	7	32	63 4–106	2 2–4 3	14 5	0 031
19	14	52	64	18 1	1 1	60	14 6–39 3	0 3–2 3	5 6	0 03[illegible]
	39	62	42	46 0	2 0	38	51 2–95 1	1 8–3 7	13 9	0 045
20	5	50	44	18 1	0 8	53	9 5–32 5	0 8–1 6	7 5	0 037
	34	66	27	57 2	1 5	29	53 1–83 7	1 4–2 3	8 6	0 022
21	5	43	63	18 1	1 2	60	11 4–25.5	0 9– 1	3 7	0 019
	44	67	23	96 6	2 1	20	94 0–149	1 9–3 4	12 7	0 025
22	2	47	49	18 8	0 9	71	18 5–27 3	0 9–1 9	8 4	0 024
	9	53	42	33 5	1 4	40	22.2–51 5	1 1–2 1	3 1	0 017
23	3	45	44	15 4	0 7	63	6 0–20 3	0 6–1 0	9 1	0 011
	12	5	48	6 0	1 2	53	16 4–35 6	0 9–2 0	5 9	0 019
24	6	46	66	17	1 1	59	20 4–26 1	1 3–1 7	5 4	0 014
	14	54	54	28 5	1 5	72	20 2–46 9	1 3–3 3	6 4	0 030

comparison between normal and sick subjects might give spurious results if the height had been affected by the pulmonary disease In relation to the gestational age the height of each infant in the present study was within normal limits (6)

In an *individual* infant a value of pulmonary dimensions deviating more than 2 S D from the expected value was regarded as abnormal When comparing *groups* of infants a paired comparison was made between obtained and expected values (calculated from the height of the infants (4)) according to standard formula (36)

RESULTS

Breathing frequency tidal volume and minute ventilation

In a few infants proper examination during rest was impossible because of periodic breathing The periodicity of the breathing pattern was abolished after exposure to 5% carbon dioxide in oxygen The values obtained at rest in these infants were obtained 2 minutes after the carbon dioxide breathing At that time the values of f V_T and V_{IE} had returned to resting values in normal infants (4)

Obtained values of f at rest were mostly within the normal range (Tables 2 and 3) Subject no 7 had an abnormally high breathing frequency at rest at the first investigation The high breathing frequency presumably reflects an excited state at the beginning of the investigation of this infant as the breathing frequency decreased during and after carbon dioxide breathing Pulmonary malfunction indicated by pathological (gf(1) and Cdyn(1) may have contributed

Tidal volume was within the normal range in most infants In subject no 21 a high V_T and a rather low f was found The V_{IE} was normal and the infant had no signs of airway obstruction

The minute ventilation was generally slightly higher than in normal infants but most values were still within the normal range Subject no 18 had a remarkably high V_{IE} but no other signs of pulmonary malfunction In subject no 1 who throughout the investigation had abnormally low values of Cdyn(1) and Gf(1) low values of V_{IE} were obtained at one occasion

During hyperventilation V_T and V_{IE} increased on the average 2 2 times The breathing frequency behaved as in normal infants This means a moderate increase in most infants Some showed a *decreasing* frequency in conjugation with carbon dioxide hyperventilation a phenomenon which is presumably due to excitation at the start of the investigation

It can thus be concluded that measurements of V_T V_{IE} and f give little information on lung function in infants of the type studied

Dynamic compliance

Obtained values of Cdyn(1) are shown in Tables 2 and 3 and in Fig 2 At the *first* investigation mechanically ventilated infants *as a group* had significantly lower Cdyn(1) than normal infants ($p<0\ 05$) Four infants (nos 1 7 9 and 10) all treated with IPPV more than 199 hours (Fig 3) had values close to or below the lower normal limit

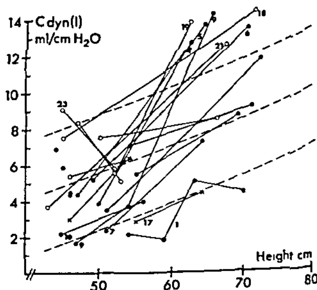


Fig 2 Dynamic compliance in relation to height The values of serially investigated infants are connected with lines The dashed lines indicate the regression line ±2 S D for normal infants (4) ● Mechanically ventilated × CPAP via an endotracheal tube ○ CPAP via a face chamber Some subjects are indicated by numbers (table 1)

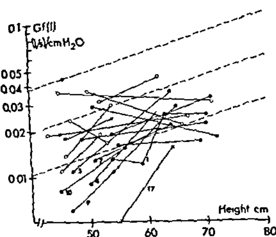


Fig 4 Functional conductance in relation to height Symbols see fig 2

loops at the initial investigation were similar to those of infants without pulmonary disease and thus evaluated as normal Eight infants had abnormal loops and low Gf(1)

Six infants with low values of Gf(1) (nos 1 4 7 9 10 and 17) had high *endexpiratory* resistance values (Table 4) and all had a rather similar respiration pattern Endinspiratory resistance was normal in most of these infants and the airflow increased normally during the first part of the expiration Later during the expiration air flow decreased in spite of a steeply increasing oesophageal pressure This must mean that the resistance was increasing very rapidly When flow became zero high endexpiratory resistance values were obtained (Fig 5 Table 4) It must be recalled that the values of endexpiratory resistance in infants with airway obstruction may to some extent reflect non homogenous properties causing *pendelluft* within the lungs (4)

Two infants with low values of Gf(1) (nos 5 and 23) showed signs of increased *endinspiratory* resistance at the first investigaion (Table 4) In subj no 23 the endinspiratory resistance appeared *infinitely* high probably due to closure of the airway at the end of the inspiration When the airways opened at a higher oesophageal pressure the expiration was performed in a normal way (Fig 6) In subj no 5 a similar but less pronounced pattern was found

In all subjects except nos 10 and 17 normal endexpiratory and endinspiratory resistance values and pressure flow loops were obtained at the reinvestigations In subject no 1 a gradual return towards normal values was found In subject no 10 an abnormal loop was obtained at reinvestigation in spite of a normal value of Rf(1) A slow decrease of airflow at high expiratory oesophageal pressure could indicate airway closure during the expiration (Fig 7) To analyse this problem 0 1 mg of adrenaline was injected through a subcutaneous needle inserted before the investigation the infant remaining asleep A decrease in endexpiratory resistance and an altered pressure flow loop were found (Fig 7) This may indicate a stabilization of previously closing airways

It can thus be concluded that pressure

Table 4 *Zero flow resistance values and Rf(1) in 8 infant with abnormal pressure flow loops*

All values in cm H_2O/(l/s) are obtained at hyperventilation The values represent one recording including eight breaths

Subj no	Age weeks	Endexspir resistance	Endinspir resistance	Functional resistance
1	14	170	30	81
	21	110	32	58
	30	99	23	43
	47	52	6	38
4	6	99	55	77
	23	21	23	33
5	10	27	53	72
	29	23	13	24
7	11	140	48	84
	29	28	35	40
9	13	300	87	170
	19	99	44	84
	40	16	26	37
10	10	320	44	110
	21	280	30	64
17	14	200	43	72
	25	320	17	58
23	3	16	∞	82
	12	29	11	36

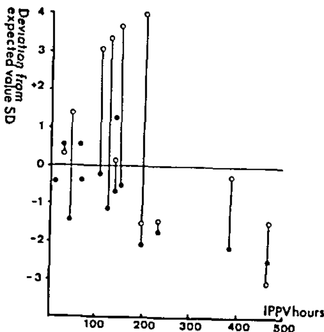


Fig 3 Deviation of dynamic compliance from expected values (expressed in S D from expected value) in relation to the duration of IPPV ● First investigation O Reinvestigation(s)

Of the 3 infants CPAP treated via an endo racheal tube 2 had normal Cdyn(1) and one no 17) had a subnormal value The in ants CPAP treated with a face chamber vere not significantly different from normal nfants although nos 22 and 23 had a *igher* Cdyn(1) than expected At rein estigation of these 2 infants which was erformed within 2 months their values vere close to those expected

The general trend observed at the rein estigation was *increasing* values of Cdyn 1) in relation to height Infants 4 5 6 and who were treated with IPPV and nos 18 9 and 21 treated with CPAP had values bove the normal range at the reinvestiga ons which were performed late i e when heir height was more than 60 cm (Fig 2) However IPPV treated subject no 1 and o 17 treated with CPAP via an endo racheal tube did not follow the trend and ill had subnormal Cdyn(1) at the rein estigations

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alues of Gf(1) are shown in Tables 2 and and Fig 4 At the *first* investigation mechanically ventilated infants *as a group* had significantly lower Gf(1) than normal infants ($p<0\ 05$) Six single infants (nos 1 4 5 7, 9 and 10) had subnormal values of Gf(1) Of the 3 infants treated with CPAP via an endotracheal tube one infant (no 17) had extremely low Gf(1) The infants treated with CPAP via a face chamber had Gf(1) values within the normal range with one exception (no 23)

At *reinvestigation* increased values of Gf(1) were found in all infants with abnormally low values at the first investigation On the other hand a tendency towards lower than expected values of Gf(1) at reinvestigation was found in most of the infants independent of the initial value at the first investigation This means that most infants examined when they were more than 60 cm high had values of Gf(1) in the low normal or slightly subnormal range

As in normal infants no correlation could be found between Gf(1) and the pulmonary elastic recoil pressure which was estimated from endexpiratory and endinspiratory pressure (4) In general there was a tendency towards low values of elastic recoil pressure which was 1 5 cm H_2O at the initial investigation and 3 cm H_2O at the reinvestigation compared to 2 (S D ±2) in normal infants

It can thus be concluded that IPPV treated infants generally had low values of Gf(1) and Cdyn(1) at the first investigation on the other hand it was generally found at the reinvestigation that irrespective of treatment the values of Gf(1) were lower but the values of Cdyn(1) higher than expected

Breathing pattern

Studies of pressure flow loops can give valuable additional information of the breathing pattern (3 4 16 21) According to the principles earlier described (3) endexpiratory and endinspiratory resistance were calculated at zero flow In 16 infants the resistance values and the pressure flow

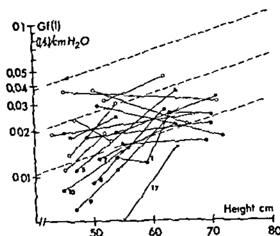


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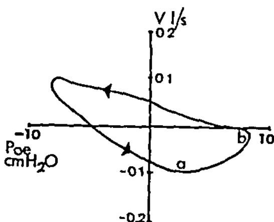


Fig 5 Pressure flow loop in subj No 1 representing a typical breathing pattern in an infant with signs of airway obstruction The arrows indicate the direction of breathing Decreasing airflow at increasing oesophageal pressure is seen during the end of the expiration (point *a* to point *b*) At the transition from expiration to inspiration the curve cuts the abscissa at a narrow angle which means a high endexpiratory resistance (3) Corresponding angle at the opposite change of flow direction shows a much lower endinspiratory resistance (Table 4) Positive flow values inspiration Negative flow values expiration

flow loops on the whole confirm the data of Gf(1) In addition it was possible better to analyse the *nature* of airway obstruction

DISCUSSION

A clearcut definition of the primary disease seems to be of the utmost importance when studying its sequelae It is likely that the healing phase and hence possible sequelae of e g a full term infant with postasphyxia syndrome is different from that of a very immature infant with respiratory insufficiency syndrome even if the treatment has been the same The diagnostic criteria described above make it possible to separate the three different pulmonary diseases included in this study though the symptoms sometimes overlap At the initial investigation the main sign of pulmonary malfunction observed was a reduction of pulmonary conductance which was obvious in 8 infants (Fig 4) Also a tendency towards low values of Cdyn(1) was noted which however was significant in only a few infants V_T V_{IE} and f were of little if any value in the evaluation of a particular infant

One prospect was that an investigation at an early stage could be of value for the evaluation of the prognosis of a particular infant In 2 (nos 5 and 23) out of the 8 infants with pathological Gf(1) the pressure flow loops showed signs of inspiratory obstruction only The reason for this abnormal breathing pattern is obscure It may indicate extrathoracic airway obstruction At reinvestigation the pressure flow loops were normal (Fig 6) None of the infants showed any clinical signs of pulmonary disease during the first year of life 6 infants had predominant expiratory obstruction 5 of them had during the first year symptoms No 1 IPPV treated for a long time because of IRDS had continuously wheezing and chest retractions at exercise during the first year but became later symptom free No 17 who received much oxygen and was CPAP treated via a tube still shows at the age of one year the same clinical picture Nos 7 9 and 10 all with RIS and IPPV treated had repeated episodes of obstructive bronchitis and pneumonia The in

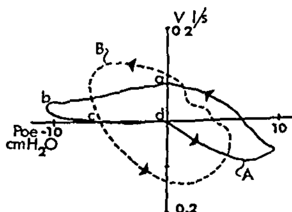


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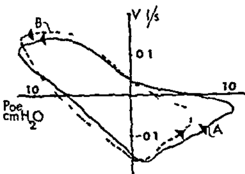


Fig 7 Pressure flow loops at the 2nd investigation in subj no 10 Loop A before adrenaline is typical of airway obstruction After administration of adrenaline (loop B) expiration is performed at a considerably lower oesophag al pressure and a normalisation of endexpira tory resistance is found *Loop A* Endexpiratory resistance 80 cm H_2O/(l/s) Rf(l) 63 cm H_2O/(l/s) Cdyn(l) 3 4 ml/cm H_2O *Loop B* Endexpiratory resistance 72 cm H_2O/(l/s) Rf(l) 39 cm H_2O/(l/s) Cdyn(l) 4 3 ml/cm H_2O

fants with normal Gf(l) had no respiratory symptoms during the first year of life

The pulmonary conductance judged together with pressure flow loops thus appears to give prognostic information

At the early reinvestigations before the in fants were 60 cm long no uniform trend was found At the late reinvestigation only the infants with continuous symptoms (nos 1 and 17) showed a pattern deviating from that of the others dominated by a low Cdyn(l) The remainder i e 12 infants showed a general tendency towards higher values than expected of Cdyn(l) and lower than ex pected Gf(l) This tendency appeared to be the same irrespective of diagnosis treat ment and the occurrence of episodical re spiratory symptoms

For a further interpretation of the above described findings some of the underlying mechanical factors must be considered At the initial investigation a low Cdyn(l) was consistently associated with a low Gf(l) This is not surprising since Cdyn(l) = V_T/ Δp where Δp is the change of oesophageal pressure between the moments when the airflow is at zero before and after inspira tion At a given V_T Δp will increase in the presence of uneven ventilation which may occur at airway obstruction when only a small fraction of V_T can pass into the most obstructive parts of the lungs This phe nomenon which is more pronounced at high breathing frequencies has been analysed in detail by Otis et al (29) Also in airway disease or at a poor elastic recoil some air ways may close during expiration thus con tributing to a low pulmonary compliance (23) Fig 7 illustrates the effect of bron chodilatation upon Δp The low values of Cdyn (l) do not necessarily indicate damage to the elastic structures of the lungs

In addition to the problems adherent to uneven ventilation the presence of atelecta tic parts in the lungs may change the appa rent elastic properties and secondarily also the resistive measures (12) There are wide possibilities for structural changes in the parenchyma effecting the pulmonary elastic properties and structural or functional changes in the airways to interact and create very complicated patterns of mechanical changes The method used does not allow a full understanding of the mechanical events within the lungs leading to changed values of Gf(l) and Cdyn(l)

In relation to normal subjects many of the infants in the present study had high values of Cdyn(l) especially at late investigations when they were higher than 60 cm The equation used for linear regression of pul monary mechanical properties on body size measured (4) is mainly based upon infants shorter than 60 cm It is likely that other correlations e g exponential are more suit able in larger infants In fact other authors (15 30) have found higher values of Cdyn(l) V_T and V_E in infants taller than 60 cm than predicted from the equation used here (4) Their values of Cdyn(l) (30) were com parable to the values obtained at the late reinvestigations in the present study The fact that most values of Cdyn(l) at the late reinvestigation are higher than predicted should therefore be evaluated with great care

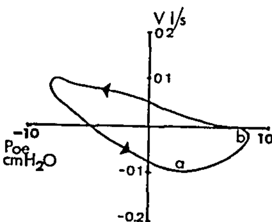


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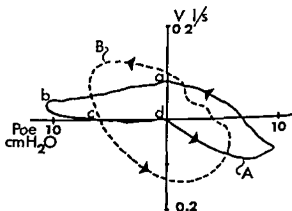


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ency towards low values of Gf(1) especially at the late reinvestigations Increased susceptibility to airway infections due to the prematurity or true sequelae from the primary disease can cause this trend

At the present time the causes of the observed abnormalities of the pulmonary mechanics cannot be definitely settled Together with other studies (32) the present results suggest that prolonged IPPV and high oxygen concentrations should be avoided The use of CPAP without intubation in the treatment of IRDS and in the facilitation of weaning from IPPV in infants with post asphyxia syndrome and RIS (2) may reduce the incidence of residual pulmonary sequelae after neonatal ventilatory insufficiency The face chamber method (1) is regarded to be so safe and convenient for CPAP treatment that its use in *early* treatment of IRDS is recommended to prevent deterioration and apnoeic spells demanding IPPV

Follow up studies of infants after neonatal respiratory insufficiency are becoming increasingly important The present method for standardized investigations and computerized calculations of pulmonary mechanics in infants has proved to be useful to indicate disturbed pulmonary mechanics Further methodological development is necessary for a more thorough understanding of the nature of the changes

ACKNOWLEDGEMENT

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REFERENCES

1 Ahlström H Jonson B & Svenningsen N W Continuous positive airway pressure with a face chamber in early treatment of idiopathic respiratory distress syndrome *Acta Paediatr Scand* 62 433 1973

2 — A new device for continuous positive airway pressure without intubation *4 e Nordiska Perinatalkongressen* Köpenhamn 6 7 april 1973

3 Ahlstrom H & Jonson B Pulmonary mechanics in infants Methodological aspects *Scand J Respir Dis* 55 129 1974

4 — Pulmonary mechanics during the first year of life *Scand J Respir Dis* 55 141 1974

5 Allen T H & Steven I M Prolonged endotracheal intubation in infants and children *Br J Anaesth* 37 566 1965

6 Babson G G & Benson R C *Primer on prematurity and high risk pregnancy* C V Mosby Company Saint Louis 1966 p 103

7 Bancelari E Garcia O L & Jesse M J Effects of continuous negative pressure on the lung mechanics in idiopathic respiratory syndrome *Pediatrics* 51 485 1973

8 Banerjee C K Girling D J & Wigglesworth J S Pulmonary fibroplasia in newborn babies treated with oxygen and artificial ventilation *Arch Dis Childh* 47 509 1972

9 Barnes N D Hull D Glover W J & Milner A D Effects of prolonged positive pressure ventilation in infancy *Lancet II* 1096 1969

10 Bomsel F Couchard M Polje J & Larroche J Cl Pulmonary sequelae of the hyaline disease Iatrogenic factors? Radiological and anatomical study *Ann Radiol* 16 70 1973

11 Bryan M H Hardie M J Reilly B J & Swyer P R Pulmonary functions studies during the first year of life in infants recovering from respiratory distress syndrome *Pediatrics* 52 169 1973

12 Butler J Caro C G Alcala R & DuBois A B Physiological factors affecting airway resistance in normal subjects and in patients with obstructive respiratory disease *J Clin Invest* 39 584 1960

13 Cederberg Å Hellsten S & Miorner G Oxygen treatment and hyaline pulmonary membranes in adults *Acta Path Microbiol Scand* 64 450 1965

14 Cook R Practical problems associated with artificial ventilation in the newborn infant. *Biol Neonate* 16 60 1970

15 Doershuk C F Downs T D Matthews L W & Lough M D A method for ventilatory measurements in subjects 1 month–5 years of age Normal results and observation in diesease *Pediatr Res* 4 165 1970

16 Fry D L & Hyatt R E A unified analysis of the relationship between pressure volume and gas flow in the lungs of normal and diseased subjects *Am J Med* 29 672 1960

17 Gaillard J L J Pathological findings in ventilated newborns *In* R H Severs & J H Huys (eds) *Physiology and pathology in the perinatal period* Leiden University Press Leiden 1971 p 145

18 Gregory G A Kitterman J A Phibbs R H Tooley W H & Hamilton W K Treatment of the idiopathic respiratory-distress *syndrome* with continuous positive airway pressure *N Engl J Med* 284 1333 1970

19 Hatch D J Prolonged nasotracheal intubation in infants and children *Lancet I* 1272 1968

The tendency towards low values of Gf(1) at late reinvestigations was significant ($p <$ 0 001) This may partially be due to bron chospasm (Fig 7) The same infant was also improved by adrenaline when it later was admitted for severe respiratory symptoms The low conductance values were not re lated to a poor elastic recoil of the lungs

Pulmonary malfunction was found to be correlated to the duration of IPPV However it cannot be stated that the IPPV *per se* is responsible for the abnormalities Infants treated with IPPV for a long time may have had a more severe degree of pul monary disease reflected in abnormal pul monary mechanics later in infancy Also among the IPPV treated infants those who showed deranged pulmonary mechanics tended to have a lower gestational age It seems likely that the more immature lungs are more susceptible to exogenous damages (from e g oxygen endotracheal tube and intermittent positive pressure) than the more mature ones Presumably however the IPPV treatment of long duration *contributes* to the pulmonary malfunction The airway obstruction after IPPV may be caused by the endotracheal tube The effect of an in dwelling tracheal tube on the mucous mem branes has been studied by several authors (5, 19 20 23 33) who reported necrosis a defective ciliary function and a decreased ability to eliminate secretion from the air ways For the development of bronchopul monary dysplasia intubation seems to be a prerequisite (32 40) In the present material only three infants not mechanically venti lated were intubated and statistical evalua tions in this small material is not reason able One of these 3 infants (no 17) had signs of bronchopulmonary dysplasia (see below) and the other 2 had values of Gf(1) and Cdyn(1) in the lower range of normal distribution (Figs 2 and 4)

The development of bronchopulmonary dysplasia is also dependent upon a high oxygen concentration (8, 26, 27, 28) Cederberg et al (13) have shown microscopic changes in the lungs in adult patients treated with oxygen concentrations as low as 40% They also found that the duration of exposi tion was of great importance The *mean* oxygen concentration in all patients mechani cally ventilated with signs of pulmonary mechanical malfunction never exceeded 40% and it is thus unlikely that oxygen toxicity is a major reason for the pulmonary mal function The single infant with persisting grave pulmonary dysfunction (no 17) was exposed to pure oxygen for 60 hours before CPAP treatment was started She was then breathing pure oxygen via an endotracheal tube for 33 hours The pressure flow loop in this infant did not show any tendency to become normal at the reinvestigation and signs of airway closure at the end of the expiration were found Oxygen toxicity may in this infant be the most important reason for the pulmonary malfunction

Most follow up studies of infants re covering from severe respiratory insufficien cy have concerned IRDS (7 10 11 25 37 38 39) In the present study the differently treated groups with IRDS are not quite comparable Infants with apnoeic spells could obviously not be treated with CPAP and it can be assumed that these infants were more ill than not apnoeic infants IPPV or CPAP treated Furthermore the *mean* gestational age was 3 weeks lower in the IPPV treated group than in the CPAP treated one Anyhow no infant treated with CPAP via the face chamber has had any clinical pulmonary symptoms or signs of grossly changed pulmonary properties

Previous studies of pulmonary function after severe IRDS have shown an in creased incidence of obstructive airway disease (38 39) The present study confirms this and indicates that other causes of neonatal ventilatory insufficiency show the same tendency

It must be recalled that irrespective of previous treatment most infants had a tend

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ELFCTRICAL POLARIZATION OF RECTAL MUCOSA AND EXCRETION OF TETRAHYDROALDOSTERONE IN PATIFNTS WITH CYSTIC FIBROSIS OF PANCREAS AND IN NORMAL SUBJECTS

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ABSTRACT Rask Madsen J Schiøtz P O Bartels U Nielsen M D and Becher Christensen F Medical Department F and the Department of Clinical Physiology Glostrup Hospital Glostrup and the Department of Pediatrics TG Rigshospitalet Copenhagen Denmark) Electrical polarization of rectal mucosa and excretion of tetrahydroaldosterone in patients with cystic fibrosis of pancreas and in normal subjects Acta Paediatr Scand 64 81 1975.—The electrical potential difference (PD) across the rectal wall was measured in 26 patients with cystic fibrosis of pancreas (CFP) and in 18 healthy subjects The PDs obtained in normal children were identical to those previously obtained in normal adults A significantly greater dispersion of the values was observed in CFP When the patients were divided into groups according to metachromasia in fibroblast cultures the mean PD was increased only in the ametachromatic group True enough this observation suggests a difference between various forms of CFP distinguished by metachromasia and thus is a further indication of the heterogeneity of the disease The greater abnormalities in metachromasia negative patients may however be due solely to the fact that these patients are more severely affected by the disease The urinary excretion of tetrahydroaldosterone in patients was within the ranges obtained in controls which excludes the possibility of secondary hyperaldosteronism as the source of increased PD No evidence was provided in favour of a basic defect in the intestinal transport of Na or Cl but K concentrations in faecal fluids of patients were significantly lower than in controls. The equilibrium concentration of K could be accounted for by simple passive diffusion suggesting that the epithelium behaved inertly with respect to this ion in CFP

KEY WORDS Absorption aldosterone biological transport cystic fibrosis of pancreas large intestine membrane potentials rectum

One of the major problems in cystic fibrosis of pancreas (CFP) is the abnormalities found in transepithelial transport of electrolytes Exact knowledge of the bioelectrical properties of colonic mucosa in patients with CFP seems important since the principal function of colonic epithelium is conservation of salt and water (6) mediated by the action of a powerful Na absorbing pump which is considered to be the source of the electrical potential difference (PD) across the colonic wall (1 3 8) Thus the PD provides a simple index for mucosal integrity (18 20) In a previous study Darby (5) reported decreased or even reversed PD in the rectum of patients suffering from CFP However the site of the reference electrode was intact skin which has a potential of its own (20) We used an intravenous electrode of saline to obtain the true transmural PD since the

20 Harrison V Heese H de V Klein M & Malan A F Prolonged endotracheal intubation in the newborn infant Br J Anaesth 39 645 1967
21 Hyatt R E The interrelationships of pressure flow and volume during various respiratory manoeuvers in normal and emphysematous subjects Am Rev Respir Dis 83 676 1961
22 Ingelstedt S Jonson B Nordström L & Olsson S-G A servo-controlled ventilator measuring expired minut volume airway flow and pressure Acta Anaesth Scand Suppl 47 7 1972
23 Jonson B Pulmonary mechanics in patients with pulmonary disease studied with the flow regulator method Scand J Clin Lab Invest 25 363 1970
24 Keuskamp D H G Comparison of ventilators for newborns and prematures In R H Severs & J H Huys (eds) Physiology and pathology in the perinatal period Leiden University Press Leiden 1971 p 165
25 Lalande J Kachaner J Dehan M & Huault G Pulmonary sequelae of neonatal respiratory distress Evolution during the first years of life Ann Radiol 16 74 1973
26 Mikity V G & Taber P Complications in the treatment of respiratory distress syndrome bronchopulmonary dysplasia oxygen toxicity and the Wilson Mikity syndrome Pediatr Clin N Amer 20 419 1973
27 Nash G Blennerhasset J B & Pontoppidan H Pulmonary lesions associated with oxygen therapy and artificial ventilation N Engl J Med 276 368 1967
28 Northway W H Rosan R C & Porter D Y Pulmonary disease following respiratory therapy of hyaline membrane disease N Engl J Med 276 357 1967
29 Otis A B McKerrow C B Bartlett R A Mead J McIlroy H B Selverstone N J & Radford E P Jr Mechanical factors in distribution of pulmonary ventilation J Appl Physiol 8 427 1955
30 Phelan P D & Williams H E Ventilatory studies of healthy infants Pediatr Res 3 425 1969
31 Prod hom L S The paediatric aspect of f asphyxia The postasphyxia syndrome In P Huntingford R W Beard F E Hytten & J Scopes (eds) Perinatal medicine London A 1970 p 131
32 Pusey V A Macphearson R J & Chernick Pulmonary fibroplasia following prolonged artif... ventilation of newborn infants Can Med Ass J 100 451 1969
33 Rasche R F H & Kuhns L R Histopathologi cal changes in airway mucosa of infants after endotracheal intubation Pediatrics 50 632 1972
34 Relier J P Mechanical complications of artificial ventilation in the newborn Biol Neonate 16 122 1970
35 Shepard F M Johnstone R B Klatte E C Burcko H & Stahlman M Residual pulmonary findings in clinical hyaline membrane disease N Engl J Med 279 1063 1968
36 Snedocor G Statistical methods Iowa State University Press Iowa 1956 p 88
37 Spoelestra A J G & Srikasibhandha S Lung mechanics in infants recovering from respiratory distress syndrome Biol Neonate 17 321 1971
38 Stahlman M Long time results of respiratory therapy Biol Neonate 16 133 1970
39 Stahlman M Hedvall G Dolanski E Faxelius G Burcko H & Kirk V A six year follow-up clinical hyaline membrane disease Pediatr Clin N Amer 20 433 1973
40 Stern L The use and misuse of oxygen in the newborn infant Pediatr Clin N Amer 20 447 1973
41 Strang L B Clinical indications for mechanical ventilation in newborn infants Biol Neonate 16 142 1970

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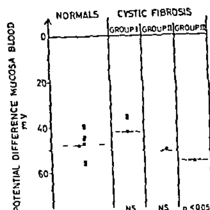


Fig. 1. The potential difference (PD) across the rectal wall in 18 normal individuals and in 26 patients with cystic fibrosis of pancreas grouped according to the distribution of metachromasia in fibroblast cultures derived from each patient (4).

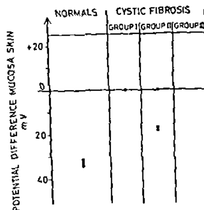


Fig. 2. The potential difference (PD) between rectal mucosa and perianal skin in 13 normal individuals and in 24 patients with cystic fibrosis of pancreas. The values are obtained by subtracting the skin–blood PD from the "true" transmural PD shown in Fig. 1.

be significantly different from one another ($p<0.01$) suggesting that the group of patients was inhomogeneous. Statistical analyses for p values were made, therefore, by comparing each of the groups I–III with the control population separately. Fig. 1 shows the individual values and the mean values of the mucosal potential relative to blood in controls and in the different groups referred to. No significant differences between mean values of the metachromasia positive groups (I and II) and normal individuals were observed. A significant augmentation of the normal negative PD was noted in the small group of patients whose fibroblasts were ametachromatic (group III, $p<0.05$).

Fig. 2 demonstrates that the potential of mucosa relative to skin was lower than the "true" transmural PD (Fig. 1) in all groups. It is also demonstrated that the PD measured by this method is abolished or even reversed in several patients, as it was originally reported by Darby (5).

No differences were found between intraluminal concentrations of Na^+ and Cl^- in the normal individuals and in the patients, while K^+ concentrations were significantly lower in the groups of patients ($p<0.01$). Table 1 shows the mean values of the transmural PD measured and the PD predicted from luminal and contraluminal concentrations of K^+, Na^+ and Cl^- by Nernst's equation. A great discrepancy between measured values and values predicted from Na^+ and Cl^- concentrations are seen both in normal individuals and in patients belonging to group II and group III. Over the relatively wide range of luminal K^+ concentrations observed (viz. 3.3–6.7 mEq/l) the PD could

Table 1. *Comparison of the potential difference observed and the potential difference predicted from luminal and contraluminal concentrations of Na^+, Cl^- and K^+ by Nernst's equation (Means $\pm$ S.D.)*

Rectal potential difference (mV)	Normals	Cystic fibrosis of pancreas: Group II ($n=11$)	Group III ($n=6$)
Observed	−49±5	−52±7	−57±9
Predicted from			
K	−66±4	−55±5	−59±5
Na	+29±5	+28±2	+29±6
Cl	−17±4	−16±5	−16±5

$p>0.1$; in all other cases p was <0.001.

serosal surface is equipotential with the blood but also measured the potential of skin relative to blood in order to compare our results with those of Darby To estimate whether the driving forces for Na^+, K^+ and Cl^- transport in rectum could be accounted for by the electrochemical gradient alone the concentrations of the named ions in stool water were measured simultaneously with the electrical PD Since a state of secondary hyperaldosteronism—a known cause of colonic hyperpolarization (7)—has been claimed to exist in patients with CFP (23) the urinary excretion of tetrahydroaldosterone and Na^+ was measured as well

MATERIAL

Studies on rectal polarization were performed on 18 healthy subjects (age 6 to 23 years) primarily admitted to hospital for minor infectious diseases or behavioural disturbances and on 31 patients with CFP (age 6 to 22 years) In 14 of the healthy subjects and in 19 patients with CFP the intraluminal equilibrium concentration of electrolytes was measured too All patients had positive sweat tests and clinical symptoms of the disease At the time of the investigation they were out patients and in a good general condition without clinical signs of active pulmonary infection None of them were currently receiving treatment with corticosteroids or diuretics but two patients had been treated with nortestosterone phenylpropionate (Durabolin®) during the last 2 or 3 months before the investigation The patients were divided into three classes according to the distribution of metachromasia in fibroblast cultures derived from each patient as described by Danes & Flensborg (4)

The studies on urinary excretion of tetrahydroaldosterone comprised 53 controls and 9 of the patients with CFP 29 subjects in the control group were school children (age 6 to 14 years) and another 6 were children in the same age group admitted to hospital for minor infectious diseases or behavioural disturbances Finally 18 adults all members of the laboratory staff were included in the study All were on a free diet without any signs of cardial renal or endocrine diseases

THEORETICAL BASIS OF ELECTRICAL AND CHEMICAL MEASUREMENTS

et movement of a charged particle is influenced by e concentration or activity gradient as well as by the lectrical PD which may exist across a given mem rane The mathematical expression which describes e relationship between the ionic concentration (*c*) tio or more precisely the activity (*a*) ratio of the ucosal (*m*) and serosal (*s*) fluids and the trans ural PD is the Nernst equation which states that

$PD = 61.5 \times \log(a_m/a_s) \approx 61.5 \times \log(c_m/c_s)$ at 37 C for monovalent ion the plasma and lumen fluids being approximately equal ionic strength Since the driv force for passive diffusion is the electrochemical gra ent across the intestinal mucosa we measured the int luminal equilibrium concentrations of Na Cl and simultaneously with the transmural PD of rectum a used the plasma values of the same electrolytes in t calculations for the serosal concentrations If the va of the observed PD conformed to the PD predicted Nernst s equation the ion was assumed to be passive transported

METHODS

Electrical measurements

The PD between rectal lumen and blood as well as th PD between the perianal skin and blood was obtaine immediately prior to the insertion of a dialysis tube a previously described in details (21) The measured valu of the PD between the luminal side and blood was compared directly with the electrical gradient as defined by the Nernst equation whereas values comparable with those of Darby (5) were obtained by subtracting the skin blood PD

Equilibrium dialysis of electrolytes

Approximately 10 cm of dialysis tube (Visking Tubing 8/32) was filled with a 10 per cent dextran solution (average molecular weight 40 000) which for the purpose of shortening the incubation period contained 75 mEq Na 100 mEq Cl and 25 mEq K per litre The tube was knotted at both ends and gently inserted into the rectum and left for 1 hour when it was removed The contents was collected for analysis to give the intra luminal equilibrium concentration of the named ions

Analytical procedures

Na and K concentrations were measured by flame photometry and Cl colorimetrically (22)

The 24 hour urinary excretion of tetrahydroaldosterone was determined by spectrophotometry after chromatographic separation (16)

Statistical methods

The values given are the means ±1 S D Statistical analyses for *p* values were by Snedecor s *F* test and by the Wilcoxon test for two samples and pair differences

RESULTS

The mean value of the transmural PD in rectum of normal children was −48 mV with a standard deviation of 5 The corresponding value based on measurements in patients with CFP was −51 mV with a S D of 10 So the variances for values obtained in normal individuals and in patients came out to

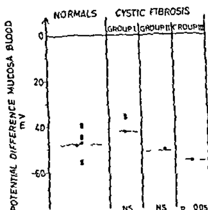


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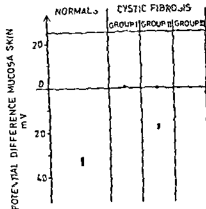


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Net movement of a charged particle is influenced by the concentration or activity gradient as well as by the electrical PD which may exist across a given membrane The mathematical expression which describes the relationship between the ionic concentration (c) ratio or more precisely the activity (a) ratio of the mucosal (m) and serosal (s) fluids and the transmural PD is the Nernst equation which states that $PD = 61.5 \times \log(a_m/a_s) \approx 61.5 \times \log(c_m/c_s)$ at 37°C for a monovalent ion the plasma and lumen fluids being of approximately equal ionic strength Since the driving force for passive diffusion is the electrochemical gradient across the intestinal mucosa we measured the intraluminal equilibrium concentrations of Na Cl and K simultaneously with the transmural PD of rectum and used the plasma values of the same electrolytes in the calculations for the serosal concentrations If the value of the observed PD conformed to the PD predicted by Nernst s equation the ion was assumed to be passively transported

METHODS

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all our patients the excretion of tetrahydro aldosterone (Fig 3) was within the normal ranges for the respective control group which excludes the possibility of secondary hyperaldosteronism The conflicting data of Simopoulos and co-workers (23) may be ex plained by the fact that their study was per formed during the hot summer season in Bethesda As regards the excretion of tetra hydroaldosterone obtained in our normal children they were about half of the values obtained in normal adults and in close agree ment with the findings of New et al (15) True enough Sole & Knorr (24) have re ported somewhat higher values but these were corrected for loss during the procedure of measurement so that the two entirely different methods turned out to agree when our recovery of 58% is taken into considera tion

Other biochemical regulators than aldoste rone may however influence the transmural PD (19) and macromolecular materials in se cretions of patients with CFP (11 13 14) might alter the configuration of membrane bound enzymes or membrane carriers in volved in transport of ions Thus it is well established that a mechanism exists in colon ic mucosa for active Na^+ absorption (1 3 8 17 21) and inversed relations between Cl^- and HCO_3^- net movements suggest the presence of an anion exchange mechanism (3 17 19) Since the concentrations of Na^+ and Cl in faecal fluids of patients were nor mal and since the PD calculated from the ratio of luminal and contraluminal concentra tions of Na and Cl^- do not conform to the values observed neither in normal individ uals nor in patients these observations pro vide no evidence in favour of a basic defect in the intestinal transport of these ions On the contrary K^+ concentrations in faecal fluids of patients are significantly lowered and can be accounted for by simple passive diffusion These findings suggest therefore that the epithelium of patients behaves in ertly with respect to this ion which is nor mally secreted into the gut lumen (19) A defect in the mechanism responsible for K^+ transfer would explain not only the findings of low intraluminal K^+ concentrations but also the tendency of raised transmural PD Further studies are needed however to de termine the validity of this concept which remains tentative

ACKNOWLEDGEMENTS

The study was supported by grants from the Danish Medical Research Council the Novo Foundation and King Christian X Foundation The technical assistance of Mrs Aa Brown Thomsen is greatly appreciated

REFERENCES

1 Cofre G & Crabbé J Active sodium transport by the colon of Bufo Marinus Stimulation by aldos terone and antidiuretic hormone *J Physiol (Lond)* 188 177 1967

2 Conover J H Conod E J & Hirschhorn K *Serumhexosaminidase* levels in cystic fibrosis *Lan cet I* 11 7 1973

3 Cooperstein I L & Hogben C A M Ionic trans fer across the isolated frog large intestine *J Gen Physiol* 42 461 1959

4 Danes B S & Flensborg E W Cystic fibrosis Cell culture studies on a Danish population *Am J Hum Genet* 23 297 1971

5 Darby C W Measurement of electrical potential difference across colonic mucosa in cystic fibrosis patients *First Annual Meeting European Working Group for Cystic Fibrosis* Stockholm 1970 abstract

6 Devroede G J & Phillips S F Conservation of sodium chloride and water by the human colon *Gastroenterology* 56 101 1969

7 Edmonds C J & Richards P Measurement of rectal electrical potential difference as an instant screening test for hyperaldosteronism *Lancet II* 624 1970

8 Grady G F Duhamel R C & Moore E W Active transport of sodium by human colon in vitro *Gastroenterology* 59 583 1970

9 Grand R J di Sant Agnese P A Talamo R C & Pallavicini J C The effects of exogenous aldos terone on sweat electrolytes I Normal subjects *J Pediatr* 70 346 1967

10 Grand R J di Sant Agnese P A Talamo R C & Pallavicini J C The effects of exogenous aldos terone on sweat electrolytes II Patients with cystic fibrosis of the pancreas *J Pediatr* 70 357 1967

11 Kaiser D Drack E & Rossi E Effect of cystic fibrosis sweat on sodium reabsorption by the normal sweat-gland *Lancet I* 1003 1970

12 Lykkegaard E & Jacobsen L Metachromatic

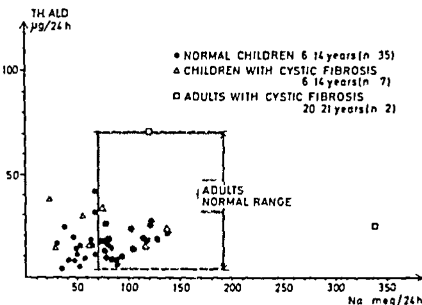


Fig 3 The relationship between 24 hour urinary excretion of Na and tetrahydroaldosterone in normal individuals and in patients with cystic fibrosis of pancreas in different groups of age

however be adequately described by the Nernst equation in the patient groups but not in the normal individuals

Fig 3 shows the urinary excretion of Na^+ and tetrahydroaldosterone in controls and in patients belonging to each of the three groups It is seen that the excretions of tetrahydroaldosterone in 7 children with CFP were all within the range obtained in normal children of the same age composition Also the Na^+ excretions were comparable in the two populations The two adult patients with CFP had both tetrahydroaldosterone excretions within the range obtained in normal adults (16) but one of them had an extraordinary high Na^+ excretion

DISCUSSION

The present measurements of the transmural PD in rectum permit the following conclusions First that the polarity of the mucosal surface is never reversed in CFP (Fig 1) as it was previously reported (5) second that the discrepancy referred to can be explained by the polarization of the skin (Fig 2) third that the mean value of rectal PD obtained in normal children (−48 mV±5) is identical to that previously obtained by the same method in normal adults (21) (−47 mV±4) and fourth that the interindividual variations in the magnitude of the true PD are considerably greater in CFP than in the normal condition

Although this study is small and preliminary the observation of increased PD in group III patients suggests a difference between various forms of CFP distinguished by metachromasia and thus is a further indication of the heterogeneity of the disease However the greater abnormalities in membrane polarization of metachromasia negative patients does not necessarily mean that this group represents a particular kind of patients but may be due to the fact that these patients seem to be more severely affected by the disease as judged by clinical lung symptoms (12) serum protein alterations (12) and serum hexosaminidase levels (2)

The reason for the observed dispersion in the results of electrical measurements in patients is nevertheless unclear but since secondary hyperaldosteronism is a well known cause of rectal hyperpolarization (7) and since it has been demonstrated that the sweat glands in patients with CFP respond normally to aldosterone (9 10) the previous findings of elevated plasma renin activity and high aldosterone secretion rates in patients with CFP (23) seemed relevant for the interpretation of our results However, in

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SINGLE INJECTION POLYFRUCTOSAN CLEARANCE IN NORMAL AND ASPHYXIATED NEONATES

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ABSTRACT Svenningsen N W (Department of Paediatrics University Hospital Lund Sweden) Single injection polyfructosan clearance in normal and asphyxiated neonates Acta Paediatr Scand 64 87 1975.—The postnatal development of the glomerular filtration rate in 36 neonates has been evaluated by the single injection polyfructosan clearance method (GFR_{PF}) by studies performed at 0–1 2–4 and 5–7 weeks of postnatal age During the first weeks of life there is a rapid postnatal increase of GFR_{PF} of almost the same magnitude in term and preterm healthy infants. Thus the postnatal development of glomerular filtration rate is more closely related to extra uterine excretory needs than to the maturational stage and gestational age at birth In the asphyxiated neonates studied the GFR_{PF} was significantly lowered in the immediate postasphyxial stage while the infants were still hypoxemic However the postnatal rise of GFR_{PF} in the time period following the postasphyxial stage was of the same order as in the non asphyxiated healthy neonates

KEY WORDS Glomerular filtration rate single injection polyfructosan renal development neonates asphyxia

In several investigations it has been stated that renal glomerular filtration evaluated by clearance measurements is low in neonates (1 2 5 7 13 14 16 24 27 33 35) Since the classical inulin clearance with sustaining continuous venous infusion and bladder catheterisation is too complicated for routine usage especially in the pediatric age group attempts have been made to simplify the technique

In recent years clearance studies with the single injection technique using different radionucleides as indicator substance have been described (3 4 15 32) However this technique is less feasible in small infants because of the irradiation and the need for multiple venous blood samples In order to overcome these disadvantages single injection techniques with non radioactive substances requiring only capillary blood samples have been introduced (10 11 12)

The aim of the present investigation is (1) to evaluate by a single injection clearance method the glomerular filtration rate in new born babies in relation to gestational and postnatal age and (2) to compare the glomerular filtration rate in asphyxiated and non asphyxiated infants in the postnatal period of life

MATERIAL

Glomerular filtration rate has been studied in neonates by repeated measurements of the single injection poly fructosan clearance rate (GFR_{PF}) The material is comprised of 10 term (group I) and 18 preterm (group II) healthy infants Their perinatal period had been uneventful i e these infants were born without signs of perinatal asphyxia and were all delivered in the vertex position In addition 8 infants with perinatal asphyxia were studied in a similar manner (group III) The infants were studied at three occasions i e at an age of 0–1 2–4 and 5–7 weeks respectively

classes in cystic fibrosis *Dan Med Bull* 1974 in press
13 Mangos J A & McSherry N R Sodium transport inhibitory factor in sweat of patients with cystic fibrosis *Science* *158* 135 1967
14 Mangos J A McSherry N R & Benke P A sodium transport inhibitory factor in the saliva of patients with cystic fibrosis of the pancreas *Pediatr Res* *1* 436 1967
15 New M I Miller B & Peterson R E Aldosterone excretion in normal children and in children with adrenal hyperplasia *J Clin Invest* *45* 412 1966
16 Nielsen M D Lund J O & Munck O Urinary excretion of tetrahydroaldosterone in normal subjects and in patients with adrenal insufficiency and Conn s syndrome *Acta Endocrinol* *71* 498 1972
17 Rask Madsen J Simultaneous measurement of electrical polarization and electrolyte transport by the entire normal and inflamed human colon during in vivo perfusion *Scand J Gastroenterol* *8* 327 1973
18 — The relationship between sodium fluxes and electrical potentials across the normal and inflamed human rectal wall in vivo *Acta Med Scand* *194* 311 1973
19 — Transepithelial ionic transport and electrical polarization in the large bowel Review *Scand J Gastroenterol* *9* 223 1974
20 Rask Madsen J & Dalmark M Decreased transmural potential difference across the human rectum in ulcerative colitis *Scand J Gastroenterol* *8* 321 1973
21 Rask Madsen J & Jensen P B Electrolyte transport capacity and electrical potentials of the normal and the inflamed human rectum in vivo *Scand J Gastroenterol* *8* 169 1973
22 Schales O & Schales S S Simple and accurate method for determination of chloride in biological fluids *J Biol Chem* *140* 879 1941
23 Simopoulos A P Lapey A Boat T F di Sant Agnese P A & Bartter F C The renin angiotensin aldosterone system in patients with cystic fibrosis of the pancreas *Pediatr Res* *5* 626 1971
24 Sole J & Knorr D Untersuchungen über die Tetrahydroaldosteron Ausscheidung im Kindesalter durch Gaschromatographie mit Elektroneneinfang detektor (ECD) *Acta Endocrinol* *70* 533 1972

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Table 1 *Polyfructosan clearance (GFR_{Ptr}) of 28 non asphyxiated neonates*
Hematocrit value at first GFR_{Ptr} measurement and age in days () at each measurement as well as formula fed are also shown Case no symbols t=term and p=preterm S D =standard deviation S E M =standard error of the mean Formula symbols A B and C (see text)

Postnatal age (weeks)				0–1	2–4	5–7	
Case no	Gest age (weeks)	Birth weight (gram)	Hematocrit	GFR_{Ptr} ml/min/1 73 m²			Formula
Term infants (Group I)							
1t boy	39	3 200	61	30 0 (2 d)	33 5 (16 d)	48 0 (36 d)	A
t boy	38	2 950	60	35 0 (2 d)	40 0 (21 d)	60 0 (42 d)	A
3t boy	39	3 100	55	28 0 (3 d)	52 0 (28 d)	–	A
4t boy	40	3 410	58	32 5 (3 d)	50 0 (21 d)	59 0 (38 d)	A
5t girl	39	3 800	52	28 5 (4 d)	34 0 (14 d)	52 0 (30 d)	A
6t girl	41	3 400	54	30 0 (4 d)	33 5 (18 d)	–	A
7t boy	41	3 100	60	32 5 (5 d)	30 5 (14 d)	40 0 (30 d)	B
8t girl	40	3 850	–	39 5 (5 d)	45 0 (20 d)	46 0 (35 d)	B
9t boy	40	2 890	54	33 0 (6 d)	–	62 5 (50 d)	B
10t boy	40	3 400	49	33 0 (7 d)	42 5 (21 d)	50 0 (37 d)	C
N	10	10	9	10	9	8	
Mean	39	3 310	55	32 2	40 1	52 1	
S D				3 21	7 34	7 27	
S E M				1 07	2 59	2 74	
Preterm infants (Group II)							
1p girl	29	1 300	53	22 5 (2 d)	28 0 (18 d)	37 0 (35 d)	A
2p girl	35	2 300	59	20 0 (2 d)	32 5 (14 d)	44 0 (40 d)	A
3p girl	34	1 940	53	19 5 (2 d)	34 0 (14 d)	38 0 (28 d)	B
4p boy	36	2 470	60	20 0 (3 d)	39 0 (23 d)	47 0 (38 d)	C
5p boy	34	2 230	49	35 0 (5 d)	37 0 (21 d)	48 0 (41 d)	A
6p boy	32	1 790	48	18 0 (5 d)	30 0 (21 d)	45 0 (40 d)	B
7p girl	31	1 760	55	25 0 (5 d)	30 0 (16 d)	40 0 (35 d)	C
	32	1 850	51	21 0 (6 d)	32 0 (19 d)	39 0 (40 d)	A
9p boy	32	1 890	59	30 0 (6 d)	32 0 (18 d)	46 0 (40 d)	B
10p boy	33	2 050	–	–	36 5 (17 d)	49 5 (36 d)	B
11p girl	35	2 430	–	–	38 4 (16 d)	51 4 (32 d)	B
12p boy	34	2 370	–	–	29 5 (11 d)	40 5 (29 d)	B
13p girl	36	2 300	–	–	30 5 (18 d)	53 5 (42 d)	C
14p boy	35	2 030	–	–	31 6 (17 d)	48 6 (35 d)	B
15p boy	33	2 080	–	–	29 0 (19 d)	39 6 (36 d)	C
16p boy	32	1 870	54	21 0 (7 d)	29 0 (23 d)	39 5 (39 d)	C
17p birl	35	2 350	–	–	34 6 (21 d)	52 0 (42 d)	C
18p boy	33	2 050	–	–	36 8 (15 d)	49 8 (32 d)	C
N	18	18	10	10	18	18	
Mean	33	2 060	54	23 2	32 6	44 9	
S D				5 10	3 45	5 20	
S E M				1 70	0 83	1 26	
P (Term higher than preterm)				<0 001	<0 05	<0 05	
P (Term 0–1 week lower than preterm 5–7 weeks)						<0 001	

RESULTS

Term and preterm healthy neonates

In Table 1 are shown the GFR_{Ptr} in term (group I) and preterm (group II) healthy infants obtained in the first week as well as at 2–4 and 5–7 weeks of postnatal life In both groups of infants there is a rapid increase of GFR_{Ptr} with increasing postnatal age At the same postnatal age the GFR_{Ptr} is significantly higher in term than in preterm infants ($p<0\ 001$ in the first postnatal week and $p<0\ 05$ at 2–4 and 5–7 weeks of age)

Clinical data of the infants in group I and II are presented in Table 1 including postnatal age at the GFR_{Pfr} measurements the hematocrit values obtained simultaneously with the first GFR_{Pfr} and the type of formula given to each infant During the first postnatal week all infants were fed human milk Thereafter either of three formulas with different protein content was given i e formula A (1 6 g protein/100 ml) formula B (2 2 g protein/100 ml) and formula C (3 8 g protein/100 ml) Regular feeding with 3–4 hours interval was started within 12 hours of age The amount of formula was increased daily with 15–20 ml per kg bodyweight After 1 week and onwards the infants were fed 140–150 ml per kg bodyweight and day

In Table 2 are presented the clinical data of the asphyxiated infants (group III) including way of delivery Apgar scores and major events in the neonatal period All these infants have sustained the type of prolonged neonatal asphyxia with hypoxemia severe acidosis and lactacidemia described as postasphyxia syndrome (23) Peroral and supplemental intravenous fluid amounting to 60 to 80 ml per kg bodyweight and day was administered during the first 72 hours and followed by increasing amounts of peroral feeding as described above All asphyxiated babies were fed human milk or formula A in the whole postnatal period It should be noted that in all infants the clearance measurements were performed regularly 3 hours after the end of the preceding meal

In all asphyxiated infants hematocrit and arterial oxygen partial pressure (Pa_{O_2}) were determined simultaneously with the clearance measurements in the first postnatal week The arterial blood samples were obtained via an umbilical artery (Argyle Umbilical Catheter Fr size 5) with the tip in the descendant aorta immediately below the renal artery In both asphyxiated and non asphyxiated infants urine bacterial cultures were performed repeatedly in order to exclude urinary tract infections

METHODS

Single injection Polyfructosan clearance procedure

Polyfructosan (25% Inutest[R] Laevasar AG Linz Austria) was given in a dosage of 0 5 ml per kg body weight as a single injection intravenously via a scalp vein Seven or eight capillary blood samples of 150 µl each were taken every 5 min during the first 20 min and thereafter every 10–15 min from 75 to 120 min following the polyfructosan injection

Standard clearance procedure

In 2 infants of group III with indwelling urine bladder catheter because of vesical paralysis a standard clearance measurement could be performed within 24 hours after a single injection clearance study It was performed as a conventional inulin clearance using polyfructosan as indicator substance After a priming dose of 0 25 ml Inutest per kg bodyweight an intravenous infusion containing 4 ml Inutest per 40 ml 5.5% glucose was administered with an infusion pump (Holter RL 174) with an infusion rate of 0 25 ml per minute After an equilibration period of 45 minutes three clearance periods around 30 minutes were used for the measurement

Calculation of clearance

The single injection polyfructosan clearance (GFR_{Pfr}) was calculated on the basis of the two-compartmental model proposed by Sapirstein et al (26) The experimental curve is resolved graphically into two exponential lines by fitting a straight line (late shallow line) through the three or four last points of the plasma disappearance curve Its intercept with the Y-axis (*A*) is determined From the first points of the experimental plasma disappearance curve are subtracted the values at corresponding time intervals on the late shallow line Thereby a second straight line (initial steep line) is obtained Its intercept with the Y-axis (*B*) is similarly determined The slopes of the initial steep line (g_1) and the late shallow line (g_2) are determined by dividing the natural logarithm of the ratio of the zero (*A* and *B* respectively) to the 10-min value on either line by 10 The value of GFR_{Pfr} is then calculated using the following formula

$$GFR_{Pfr}(ml/min) = \frac{P\ g_1\ g_2}{A\ g_2 + B\ g_1}$$

P is the dose of polyfructosan in mg injected GFR_{Pfr} is corrected to 1 73 m² body surface A desk model computer (Olivetti Programma 101) was programmed to perform the calculations based on these equations

The standard clearance was calculated according to the classical formula

$$GFR_{Pfr}(ml/min) = \frac{U_{Pfr}\ V}{P_{Pfr}}$$

U_{Pfr} and P_{Pfr} are concentrations of polyfructosan in urine and plasma and *V* urine flow in ml per minute The clearance values are correlated to 1 73 m² body surface

Laboratory analysis

Polyfructosan was determined according to the method of Heyrowsky (18) with a modification for capillary blood necessitating only 50 µl plasma for each analysis When polyfructosan had been administered in a glucose infusion as during the standard clearance procedure (vide supra) 100 µl of a 25% yeast suspension was added to each sample in order to eliminate any interferance of glucose Capillary blood hematocrit was measured after centrifugation at 5 000 rpm for 10 min Determination of arterial Pa_{O_2} and acid base status was performed with an Eschweiler pH and Blood Gas Analyzer (Eschweiler & Co Kiel BRD) as earlier described (30) Blood lactate was determined enzymatically (29)

Table 2 *Clinical data of 8 asphyxiated neonates (group III)*

Case number symbols a=asphyxiated Pa_{O_2} (100% O_2)=Arterial oxygen pressure after breathing 100% oxygen for 10 minutes IRDS=idiopathic respiratory distress syndrome CPAP=Continous positive airway pressure via face chamber

Case	Gest age (weeks)	Birth weight (gram)	Delivery	Apgar score 1 – 5 – 10 min	Neonatal period
1a girl	42	3 400	Intra uterine asphyxia Cesarean section	6 – 7 – 6	Postasphyxiasyndrome Pulmonary atelectases severe acidosis blood pH 6 95 at 2 hours Intubation and ventilation for one hour Oliguria with 4 ml urine during the first 48 hours Survived
2a girl	38	2 830	Intra uterine asphyxia Vertex presentation	9 – 8 – 7	Persisting postnatal cyanosis Pa_{O_2} (100% O_2) 60 mmHg at 24 hours Heart catheterisation normal Normoxemia after correction of acidosis Survived
3a boy	37	3 400	Vertex presentation	4 – 6 – 7	Postasphyxiasyndrome Pulmonary bilateral massive atelectases Pa_O below 40 mmHg for 4 days Ventilator treatment for 11 days Survived
4a boy	39	2 130	Breech presentation	3 – 7 – 9	Postasphyxia syndrome with pulmonary atelectases Persisting hypotonia PaO_2 below 45 mmHg 2 days Survived
5a girl	3[illegible]	1 800	Vertex presentation	4 – 6 – 8	IRDS Pa_{O_2} (100%) O_2) 50 mmHg at 2[illegible] hours Ventilator treatment followed by CPAP for 4 days Survived
6a boy	40	2 950	Precipitate delivery Vertex presentation	8 – 5 – 7	Postasphyxia syndrome Pulmonary massive atelectases Pa_{O_2} (100% O_2) sive mmHg at 24 hours Pa_{O_2} below 45 mmHg for 2 days Ventilator treatment for 3 days Survived
7a boy	41	4 490	Vertex presentation	4 – 7 – 8	Persisting cyanosis and hyptonia Severe acidosis blood pH 6 90 lactate 7 5 mmol/l Cardiac failure Oliguria with 10 ml urine in 48 hours Survived
8a girl	42	3 400	Vertex presentation	6 – 8 – 6	Postasphyxiasyndrome Polmonary massive atelectases Pa_{O_2} (100% O_2) 29 mmHg blood pH 6 82 lactate 9 5 mMol/l at 12 hours Ventilator treatment for 3 days followed by CPAP for 2 days Survived

measurements in the first week of life (Tables 1 and 3) The hematocrit values ranged between 48 and 62 all being within the normal range at this age (20) Consequently major changes in blood volume which might have effect upon glomerular filtration rate (22) presumably did no exert any influence upon GFR_{Pfr} of these infants

Protein intake

No correlation between the postnatal rise of GFR_{Pfr} and the protein intake could be observed in the present investigation However the small number of infants within each dietary group does not allow any definite conclusions regarding dietary influences upon postnatal development of glomerular filtration rate

DISCUSSION

Measurement of glomerular filtration rate

In studies of the glomerular filtration rate the technique employed must be reliable

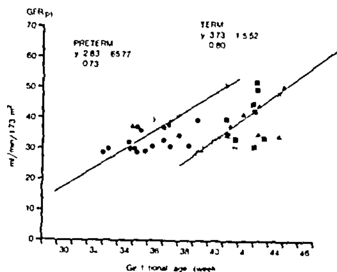


Fig 1 Single injection Polyfructosan clearance GFR$_{Pfr}$) in relation to gestational age in term preterm and asphyxiated neonates Regression equation correlation coefficient and its significance for healthy infants from 1 to 7 weeks of postnatal age
Term $y=3\ 7283x-115\ 5212$ $r=0\ 80$ $P<0\ 001$
Preterm $y=2\ 8281x-65\ 7664$ $r=0\ 73$ $P<0\ 001$
(y=GFR in ml/min/1 73 m² x=gestational age in weeks)

Symbols		*0–1*	*2–4*	*5–7 postnatal weeks*
Term	infants (group I)	□	■	□
Preterm	infants (group II)	○	●	○
Asphyxiated	infants (group III)	△	▲	△

Fig 1 shows the GFR$_{Pfr}$ in relation to gestational age There is in all infants a conspicuous increase with increasing gestational age Thus the postnatal rise is of almost the same magnitude in term and preterm infants The slope of the regression line is only slightly lower in preterm infants (=2 8281 r=0 73) than in term infants (b=3 7283 r=0 80)

The postnatal rise is further illustrated by comparing preterm infants 5–7 weeks old and term infants 0–1 week of postnatal age Although of the same gestational age (38 to 42 weeks of gestation) the GFR$_{Pfr}$ values are significantly higher in preterm than in term infants ($p<0\ 001$ Table 1)

Asphyxiated neonates

The GFR$_{Pfr}$ values of the asphyxiated infants of group III are presented in Table 3 and Fig 1 In the first postnatal week, i e in the immediate postasphyxial stage, the GFR$_{Pfr}$ is significantly lower in these infants than in the non asphyxiated term infants of the same postnatal age ($p<0\ 001$) It should be noted that the Pa$_{O_2}$ measured simultaneously with the first GFR$_{Pfr}$ ranged between 35 and 65 mmHg with a mean value of 49 2 mmHg These Pa$_{O_2}$ values are all below the normal range limit for Pa$_{O_2}$ in healthy infants of the same postnatal age (20) Thus the asphyxiated infants were still moderately hypoxemic at the first GFR$_{Pfr}$ measurement As shown in Fig 2 there is a direct correlation between GFR$_{Pfr}$ and Pa$_{O_2}$ measured in samples from the descendant aorta

After the first week of life there is a rapid enhancement of GFR$_{Pfr}$ in the asphyxiated infants reaching values almost equal to those found in healthy infants at 5–7 weeks of age (Fig 1)

Blood volume

Capillary blood hematocrit was determined in all infants simultaneously with GFR$_{Pfr}$

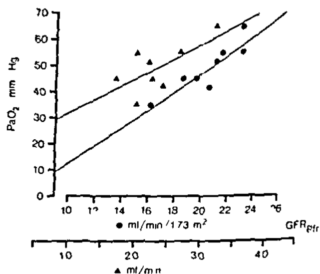


Fig 2 Single injection Polyfructosan clearance (GFR$_{Pfr}$) in relation to Pa$_{O_2}$ in aorta descendant in asphyxiated neonates (group III) at 2 to 8 days of postnatal age Regression equation correlation coefficient and its significance
$y=3\ 543x_1-22\ 9467$ $r=0\ 89$ $p<0\ 001$
$y=13\ 2910x_2+18\ 7305$ $r=0\ 65$ $p<0\ 05$
(y=Pa$_{O_2}$ mmHg in aorta descendent x_1=GFR$_{Pfr}$ in ml/min/1 73 m² x_2=GFR$_{Pfr}$ in ml/min)
Symbols x_1 ● x_2 ▲

Table 4 *Compilation of literature data of glomerular filtration in normal neonates in the first weeks of life*
N=number of infants studied S D =standard deviation

Investigator	Technique	Infants	N	Age	GFR (ml/min/1 73 m²)	
					Mean±S D	Range
Dean & McCance 1947 (7)	Inulin (constant infusion)	Term	8	2–8 d		16 3–44 6
Barnett et al 1948 (2)	Inulin (single injection + constant infusion)	Preterm	21 21	3–4 d 9–13 d		3 4–5 4 ml/min 3 3–6 6 ml/min
Vesterdal & Tudvad 1949 (33)	Inulin (subcutaneous injection)	Term Preterm	6 5	2–13 d 2–10 d		12 0–38 0 12 0–31 0
Friedenszick 1957 (13)	Inulin (constant infusion)	Term	11	21–56 d		37 7–50 0
Friedberg & Jung 1957 (14)	Inulin (constant infusion)	Preterm <2 kg Preterm >2 kg Term	4 5 19	1 d 1 d 1 d	17 3±6.8 27 [illegible]±6 6 25 5±9 9	
Winberg 1959 (35)	Creatinine (24 hour endogenous)	Term Term	3 2	4–8 d 9–13 d		27 0–63 0 38 0–54 0
Rohwedder 1965 (24)	Inulin (constant infusion)	Term	10	3–[illegible]8 d	41 4±7 3	
Oh Oh & Lind 1966 ([illegible])	Inulin (constant infusion)	Term	14 29 6 18	1–12 hrs[a] 1–1[illegible] hrs[b] 2–5 d 2–5 d[b]	20 3 28 6 33 0 37 5	12 0–29 0 10 0–51 0 17 0–44 6 13 0–75 0
Aperia et al 1972 (1)	Inulin (single injection)	Term	5	3–15 d		20 0–34 0
Sertel & Scopes 1973 (27)	Creatinine (short endogenous)	Term	17 1[illegible]	1 d 6 d		4 0–48 0 16 0–90 0
Broberger 1973 (5)	Inulin (single injection)	Term	11	4–14 d		26 0–5[illegible] 0
Present investigation	Polyfructosan (single injection)	Term	10 9 8	2–[illegible] d 2–4 w 5–7 w	32 2±3 2 40 1±7 3 52 1±7 2	28 0–39 5 30 5–52 0 40 0–62 5
		Preterm	10 18 18	2–7 d 2–4 w 5–7 w	23 2±5 1 32 6±3 4 44 9±5.2	18 0–35 0 28 0–39 0 37 0–53.5

[a] Early clamped
[b] Late clamped

ments could be performed only in two infants in the first week of life Single injection and standard clearance measurements gave the following results in one infant 16 0 and 19 5 ml/min/1 73 m² and in another infant 20 5 and 22 5 ml/min/1 73 m² respectively (case nos 1*a* and 8*a* in Table 3) Thus the single injection method only slightly underestimated glomerular filtration rate according to the standard clearance technique Similar observations have been made in another study of newborn infants (5)

Polyfructosan clearance in healthy neonates

The values of GFR_{PF} obtained in the present investigation are similar to those obtained in early infancy in investigations ear

Table 3 *Polyfructosan clearance (GFR_{Pfr}) of 8 asphyxiated neonates (group III)*
Hematocrit and aorta Pa_{O_2} at first GFR_{Pfr} measurement as well as age in days at each measurement () are also shown
Case no symbols a=asphyxiated neonate n s =not significant

Postnatal age (weeks)			0–1		2–4	5–7
Case no	Gest age (weeks)	Hematocrit	Pa_{O_2} mmHg	GFR_{Pfr} ml/min/1 73 m²		
1a	42	56	35 0	16 0 (2 d)	38 0 (19 d)	47 0 (37 d)
2a	38	52	52 0	21 0 (2 d)	29 0 (22 d)	–
3a	37	62	45 0	19 5 (3 d)	–	38 0 (29 d)
4a	39	58	55 0	21 5 (4 d)	41 0 (23 d)	50 5 (47 d)
5a	32	60	45 0	18 5 (4 d)	38 5 (17 d)	50 0 (39 d)
6a	40	51	55 0	23 0 (5 d)	44 5 (23 d)	–
7a	41	54	65 5	23 0 (5 d)	35 0 (15 d)	67 0 (35 d)
8a	42	49	42 0	20 5 (8 d)	30 0 (16 d)	45 0 (32 d)
N	8	8	8	8	7	6
Mean		55	49 2	20 3	36 5	49 5
S D			9 3	2 20	5.22	8 81
S E M			3 3	0 83	2 13	3 94
P (term higher than asphyxiated)				<0 001	n s	n s

and convenient The conventional inulin clearance is generally considered the most accurate method However the standard technique with continuous intravenous infusion and urine collection by means of bladder catheterisation presents several problems especially in small infants and when frequent determinations are needed (9 25 36) Endogenous creatinine clearance has been advocated as an alternative Yet multiple errors are involved in this method mainly related to the variability in the rate of tubular transport of creatinine and to incomplete urine collection (8 27 35) A marked increase of creatinine clearance above inulin clearance values has also been observed during water diuresis (6)

In order to overcome the abovementioned difficulties single injection techniques have been introduced with estimation of the glomerular filtration rate from the plasma disappearance curve of an indicator substance Several authors have shown that such methods correlate well with the conventional inulin clearance method (5, 9 25 28, 32) The exposure to irradiation when using radionucleides has been a major obstacle for using such indicator substances in small infants This can be avoided by using inulin or an analogue of inulin like polyfructosan Besides being cold soluble and alkaline stable polyfructosan is preferable to inulin because of smaller and more uniform molecular size i e 3 000 instead of 5 000 for inulin (11)

The glomerular filtration rate is calculated according to a conventional two compartmental system as presented by Sapirstein et al (26) either after a short term infusion (11 16) or a single injection dose (1 5 10 12) If the two compartmental system is applied with caution i e not used in extreme situations such as large edema or severe renal insufficiency the single injection technique is considered to give sufficient precise results (10 11 15 28)

Comparison between conventional continuous infusion clearance and single injection clearance methods has shown satisfactory results with correlation coefficients above 0 90 (5 28 34) In the present investigation comparative clearance measure

Donath A The simultaneous determination in children of glomerular filtration rate and effective renal plasma flow by the single injection clearance technique *Acta Paediatr Scand 60* 51? 1971

Earon J Blaufox M D Edelmann C M Jr & Spitzer A A simplified method for estimating GFR from the blood disappearance of inulin *Pediatr Res 7* 413 1973

Falbriard A & Zender R E Mesure de la fonction glomérulaire par la décroissance plasmatique d une substance analogue á l/inuline (Polyfructosan S) *Nephron 1* 277 1964

Favre H Zender R & Falbriard A Decroissance plasmatique du Polyfructosan S chez l homme après injection unique *Helv Physiol Acta 26* 79 1968

Friederiszick F K Nieren Clearance Untersuchungen im Kindesalter *Ann Paediat* Suppl *57* 1957

Friedberg W & Jung R Die Nierenfunktion beim Neugeborenen *Arzt Forsch 11* 306 1957

Garnett E S Parsons V & Veall N Measurement of glomerular filtration rate in man using a 51 Cr/edetica id complex *Lancet 1* 818 1967

Geisert J Willard D Sacrez R Signist D & Mack G Clearance glomerulaire et fraction de filtration chez l enfant *Arch Fr Pediatr 26* 1?5 1969

' Gruskin A B Edelmann C M Jr & Yuan S Maturational changes in renal blood flow in piglets *Ped atr Res 4* 13 1970

3 Heyrowsky A A new method for the determination of inulin in plasma and urine *Clin Chim Acta 1* 470 1956

? Horster M & Valentin H Postnatal development of renal function micropuncture and clearance studies in the dog *J Clin Invest 50* 779 1971

0 Koch G & Wendel H The adjustment of arterial blood gases and acid–base balance in the normal newborn infant during the first weeks of life *Biol Neonate 12* 136 1968

!1 McCance R A & Widdowson E M The influence of events during the last few days in utero on tissue destruction and renal function in the first two days of independent life *Arch Dis Childh 29* 495 1954

2 Oh W Oh M A & Lind J Renal function and blood volume in newborn infants related to placental transfusion *Acta Paediatr Scand 56* 197 1966

23 Prod'hom L S The paediatric aspect of fetal asphyxia the postasphyxia syndrome *Proc 2nd Eur Congr Perinatal Med* London April 1970 p 131

?4 Rohwedder H J Über die Clearance in der ersten Lebenszeit *In* Linneweh (ed) *Fortschritte der Pädologie II* Springer Verlag Berlin 1965 p 218

25 Sakai T Leumann E P & Holliday M A Single injection clearance in children *Pediatrics 44* 905 1969

?6 Sapirstein L A Vidt D G Mandel M J & Hanusek G Volumes of distribution and clearances of intravenously injected creatinine in the dog *Am J Physiol 181* 330 1955

?7 Sertel H & Scopes J Rates of creatinine clearance in babies less than one week of age *Arch Dis Childh 48* 717 1973

28 Silkalns G I Jeck D Earon J Edelmann C M Jr Chervu L R Blaufox M D & Spitzer A Simultaneous measurement of glomerular filtration rate and renal plasma flow using plasma disappearance curves *J Pediatr 83* 749 1973

29 Svenningsen N W & Siesjo B K Cerebrospinal fluid lactate/pyruvate ratio in normal and asphyxiated neonates *Acta Paediatr Scand 61* 117 1972

30 Svenningsen N W Renal acid–base titration studies in infants with an without metabolic acidosis in the postneonatal period *Pediatr Res 8* 659 1974

31 Torrado A & Gautier E Renal function in hyaline membrane disease *Proc Eur Soc Ped Nephr* Helsinki 1969 p 15

3? Truniger B Donath A & Koppeler A Simplified clearance techniques The single injection method and its modifications *Helv Med Acta 34* 116 1968

33 Vesterdal J & Tudvad F Studies on the kidney function in premature and fullterm infants by estimation of the inulin and para aminohippurate clearances *Acta Paediatr Scand 37* 429 1949

34 Vogeli B Riedwyl H Donath A & Oetliker O Comparison of glomerular filtration rate and effective renal plasma flow determinations obtained by a single injection technique and by means of a standard clearance technique in children *Acta Paediatr Scand 60* 528 1971

35 Winberg J The 24 hour true endogenous creatinine clearance in infants and children without renal disease *Acta Paediatr Scand 48* 443 1959

36 Zender R Vuagnat P & Falbriard A Etude statistique des causes d erreur dans la mesure d une clearance renale conventionelle *Clin Chim Acta 20* 85 1968

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lier reported in the literature and compiled in Table 4 The results of the present investigation show that there is a considerable postnatal increase of GFR_{Pfr} during the first 5 to 7 weeks of postnatal life Although this increase initially is more gradual and less steep in preterm infants there is anyhow a significant difference by comparison between preterm infants 5–7 weeks old and term infants 1 week old but of the same gestational age The GFR_{Pfr} values are significantly higher in the preterm than in the term infants indicating that the postnatal development of glomerular filtration rate is related not only to maturational stage and gestational age at birth but more closely to extra uterine excretory needs This is in agreement with observations in animal studies showing that the postnatal rise of glomerular filtration is to a large extent functionally regulated and not merely follows the anatomical growth of the kidney (17 19)

Polyfructosan clearance in asphyxiated neonates

The GFR_{Pfr} of asphyxiated infants was found to be decreased in the immediate post asphyxial stage This lowering of the GFR_{Pfr} is probably related to the hypoxia still present in most of these infants at the first GFR_{Pfr} measurement (Table 3 and Fig 2) This is in accordance with observations made by McCance and Widdowson (21) who found a reduced glomerular filtration rate a poor urea clearance and a low urine volume in fullterm and postmature infants after prolonged and difficult labour A significant decrease in urinary output directly correlated to the degree of hypoxemia has been found in infants with hyaline membrane disease studied during the first 48 hours of life (31) In healthy piglets there is from birth to 45 days of age an increase in renal blood flow in parallel to rising glomerular filtration rate (17) During postnatal adaptation the glomerular filtration is apparently under close hemodynamic control in order to balance the tubular reabsorption capacity (17 19) The close correlation between glomerular filtration rate and aorta Pa_{O_2} observed in the present investigation (Fig ?) indicates that the low GFR_{Pfr} in the asphyxiated neonates possibly is a consequence of renal hemodynamic disturbances secondary to asphyxia

Besides the impairment of glomerular filtration postasphyxial derangement of renal tubular function must also be taken into consideration This will contribute to functional glomerulo tubular imbalance in the postasphyxial stage as well Tubular function studies have therefore been performed in the asphyxiated infants of the present material These results will be presented separately

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REFERENCES

1 Aperia A Broberger O Thodenius K & Zetterstrom R Renal response to an oral sodium load in newborn full term infants *Acta Paediatr Scand 61* 670 1972
2 Barnett H L Hare K McNamara H & Hare R Measurement of glomerular filtration rate in premature infants *J Clin Invest 27* 691 1948
3 Blaufox M D Frohmuller H G W Campbell J C Utz D C Orvis A L & Owen C A A simplified method of estimating renal function with Iodohippurate I 131 *J Surg Res 3* 127 1963
4 Blaufox M D & Merrill J P Simplified hippuran clearance *Nephron 3* 122 1966
5 Broberger U Determination of glomerular filtration rate in the newborn *Acta Paediatr Scand 62* 625 1973
6 Calcagno P L Rubin M I & Weintraub D H Studies on the renal concentrating and diluting mechanisms in the premature infant *J Clin Invest 33* 91 1954
7 Dean B E A & McCance R A Inulin Diodone Creatinine and urea clearance in newborn infants *J Physiol 106* 431 1947
8 Dodge W F Travis L B & Daeschner C W Comparison of endogenous creatinine clearance with inulin clearance *Am J Dis Child 113* 683 1967

9 Donath A The simultaneous determination in children of glomerular filtration rate and effective renal plasma flow by the single injection clearance technique *Acta Paediatr Scand 60* 512 1971

10 Earon J Blaufox M D Edelmann C M Jr & Spitzer A A simplified method for estimating GFR from the blood disappearance of inulin *Pediatr Res 7* 413 1973

11 Falbriard A & Zender R E Mesure de la fonction glomerulaire par la decroissance plasmatique d une substance analogue á l/inuline (Polyfructosan S) *Nephron 1* 277 1964

12 Favre H Zender R & Falbriard A Decroissance plasmatique du Polyfructosan S chez l homme apres injection unique *Helv Physiol Acta 26* 79 1968

13 Friederiszick F K Nieren Clearance Untersuchungen im Kindesalter *Ann Paediat* Suppl 57 1957

14 Friedberg W & Jung R Die Nierenfunktion beim Neugeborenen *ArzlForsch 11* 306 1957

15 Garnett E S Parsons V & Veall N Measurement of glomerular filtration rate in man using a 51 Cr/edeticacid complex *Lancet I* 818 1967

16 Geisert J Willard D Sacrez R Sigrist D & Mack G Clearance glomerulaire et fraction de filtration chez l enfant *Arch Fr Pediatr 26* 125 1969

17 Gruskin A B Edelmann C M Jr & Yuan S Maturational changes in renal blood flow in piglets *Pediatr Res 4* 13 1970

18 Heyrowsky A A new method for the determination of inulin in plasma and urine *Clin Chim Acta 1* 470 1956

19 Horster M & Valentin H Postnatal development of renal function micropuncture and clearance studies in the dog *J Clin Invest 50* 779 1971

20 Koch G & Wendel H The adjustment of arterial blood gases and acid–base balance in the normal newborn infant during the first weeks of life *Biol Neonate 12* 136 1968

21 McCance R A & Widdowson E M The influence of events during the last few days in utero on tissue destruction and renal function in the first two days of independent life *Arch Dis Childh 29* 495 1954

22 Oh W Oh M A & Lind J Renal function and blood volume in newborn infants related to placental transfusion *Acta Paediatr Scand 56* 197 1966

23 Prod'hom L S The paediatric aspect of fetal asphyxia the postasphyxia syndrome *Proc 2nd Eur Congr Perinatal Med* London April 1970 p 131

24 Rohwedder H J Über die Clearance in der ersten Lebenszeit *In* Linneweh (ed) *Fortschritte der Padologie II* Springer Verlag Berlin 1965 p 218

25 Sakai T Leumann E P & Holliday M A Single injection clearance in children *Pediatrics 44* 905 1969

26 Sapirstein L A Vidt D G Mandel M J & Hanusek G Volumes of distribution and clearances of intravenously injected creatinine in the dog *Am J Physiol 181* 330 1955

27 Sertel H & Scopes J Rates of creatinine clearance in babies less than one week of age *Arch Dis Childh 48* 717 1973

28 Silkalns G I Jeck D Earon J Edelmann C M Jr Chervu L R Blaufox M D & Spitzer A Simultaneous measurement of glomerular filtration rate and renal plasma flow using plasma disappearance curves *J Pediatr 83* 749 1973

29 Svenningsen N W & Siesjö B K Cerebrospinal fluid lactate/pyruvate ratio in normal and asphyxiated neonates *Acta Paediatr Scand 61* 117 1972

30 Svenningsen N W Renal acid–base titration studies in infants with an without metabolic acidosis in the postneonatal period *Ped atr Res 8* 659 1974

31 Torrado A & Gautier E Renal function in hyaline membrane disease *Proc Eur Soc Ped Nephr* Helsinki 1969 p 15

32 Truniger B Donath A & Koppeler A Simplified clearance techniques The single injection method and its modifications *Helv Med Acta 34* 116 1968

33 Vesterdal J & Tudvad F Studies on the kidney function in premature and fullterm infants by estimation of the inulin and para aminohippurate clearances *Acta Paediatr Scand 37* 429 1949

34 Vogeli B Riedwyl H Donath A & Oetliker O Comparison of glomerular filtration rate and effective renal plasma flow determinations obtained by a single injection technique and by means of a standard clearance technique in children *Acta Paediatr Scand 60* 528 1971

35 Winberg J The 24 hour true endogenous creatinine clearance in infants and children without renal disease *Acta Paediatr Scand 48* 443 1959

36 Zender R Vuagnat P & Falbriard A Etude statistique des causes d erreur dans la mesure d une clearance rénale conventionelle *Clin Chim Acta 20* 85 1968

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THE IMMUNE RESPONSE TO URINARY TRACT INFECTIONS IN CHILDHOOD

I Serological Diagnosis of Primary Symptomatic Infection in Girls by Indirect Hemagglutination

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ABSTRACT Jodal U (Institute of Medical Microbiology and Department of Pediatrics University of Goteborg Goteborg Sweden) The immune response to urinary tract infections in childhood I Serological diagnosis of primary symptomatic infection in girls by indirect hemagglutination Acta Paediatr Scand 64 96 1975 —Determination of antibodies to E coli O antigen by indirect hemagglutination for detection of acute pyelonephritis was investigated in sequences of sera from 94 girls with their first known attack of symptomatic urinary tract infection Using O antigen from the infecting bacterial strain or a standard strain increased antibody titres were found among pyelonephritis patients in 89% in unreduced sera (mainly 19 S antibodies) and in 81% in reduced sera (mainly 7 S antibodies) compared to a control material of 643 healthy children Significant changes of these antibody titres occurred in 74 and 39% respectively Analysis of maximal titres as well as titre changes in both unreduced and reduced sera showed diagnostically significant changes in two or more of these four parameters in 89% of patients with pyelonephritis In contrast one parameter with such changes was found among the cystitis patients in only 5% Employing a pool of 8 common O antigens increased antibody titres were detected in 76% of patients with pyelonephritis A larger pool of 68 antigens was no better and had a low capacity to detect 7 S antibodies Optimal time for blood sampling was 2 to 3 weeks after onset of symptoms

KEY WORDS E coli immunology urinary tract infection kidney concentrating ability infants children

Analysis of the serum antibody response to urinary tract infection (UTI) by agglutination techniques has been found useful in differentiating acute pyelonephritis from acute cystitis (5 19 21 23 25 27) However other studies have not demonstrated a relationship between elevated titres and infection of the renal parenchyma (8 18) These variable results may be caused by the use of different techniques but also by differences in the patient material UTI is not a homogenous disease and several factors such as sex age malformations and neurologic abnormalities are known to influence the frequency and severity of UTI which can result in differences in the immune response In particular the antibody response to recurrent infections would be expected to differ from that to the first infection Whereas 19 S IgM antibodies dominate the immune response against the O antigen in primary infections it has recently been observed that 7 S IgG antibodies also appear—especially in recurrent infections (10 11 24) Most antibody studies in patients with UTI have been performed by agglutination methods which favour the IgM antibodies (20) However treatment of serum with 2 mercaptoethanol

(ME) allows measurement also of reduction resistant 7 S antibodies (mainly IgG) by indirect hemagglutination

The aim of the present study was to investigate the usefulness of determination of reduction sensitive and resistant serum antibodies for level diagnosis in a clinically well characterized patient material consisting of girls with their first attack of acute UTI In order to define the optimal time of sampling and to test the value of analysis of titer changes of reduction resistant as well as sensitive antibodies during the course of the UTI several serum samples from each patient were analysed Preparation of O antigen from the urinary isolate in each patient for the red cell sensitization poses considerable problems in routine practice Thus this study also included an evaluation of the usefulness of pools of the most common antigens as suggested by Andersen (2)

MATERIAL AND METHODS

Patients

The patient population consisted of 94 girls age ? months to 15 years presenting at the Children s Hospital Goteborg with their first known symptomatic UTI and whose urine cultured significant (at least 10^5/ml) *E coli* The requirement for this bacterial species resulted in the exclusion of only a few patients with cystitis caused by *Proteus* bacteria

The diagnostic criteria for pyelonephritis were fever of at least 38 5°C transiently decreased renal concentrating capacity in relation to age (6) and increased sedimentation rate (3 mm/h or more with the microtechnique) (10 ?7) All of these criteria were fulfilled for 35 of the aforementioned 54 pyelonephritis patients while for the remaining 19 girls either the concentrating capacity (1 girls) or the sedimentation rate (7 girls) was not determined in the acute phase Results were similar for the girls with all of the diagnostic criteria for pyelonephritis and for those in whom one of the tests had not been performed Therefore the results for the pyelonephritis group are presented together

The criteria for the diagnosis of cystitis included symptom of burning and frequency but absence of abdominal or flank (loin) pain temperature not exceeding 38 0°C renal concentrating capacity >815 mOsm/l and sedimentation rate <.5 mm/h

The age range for the pyelonephritis patients was ? months to 9 years compared to 1 1/? to 15 years for the group with acute cystitis In 43 of the girls with pyelonephritis including those 17 under the age of 1 year and those 17 developing a recurrence during a one year follow up period intravenous pyelography and micturating cysto urethrography were performed No cases of obstructive uropathy were found

All of the patients were successfully treated with an antibiotic for at least 10 days usually with sulphafurazol 00 mg/kg/day While most were treated as out patients 30 of the 54 girls with pyelonephritis were hospitalized for 3–10 days Three girls had a symptomatic recurrence within 2 weeks after the therapy had been completed all other recurrences occurred after more than 1 month Serum samples were obtained at least once within 5–8 days after onset of symptoms and then on 1–3 other occasions during the following 2 months These samples were frozen in aliquots at –?0°C without delay and thawed immediately before testing The use of aliquots was motivated by the experience that repeated freezing and thawing will appreciably reduce antibody titres especially of the 19 S antibodies

Controls

The sera from 643 healthy children (313 females) seen at welfare centers and at four schools were used to establish antibody titres in the normal population These control subjects had neither a history of UTI nor bacteriuria by quantitative culture

Serological methods

The *E coli* urinary isolates from the patients were O grouped by direct bacterial agglutination employing specific antisera (17) Sera from patients and controls were titrated by indirect hemagglutination performed as described earlier (?7) but employing a micropipetter (Oxford 0 0?5 ml) for the serial dilutions In 1430 duplicate titrations this technique resulted in a standard deviation of 0 58 titre steps Titres were expressed as –log2 For sensitization was used the O antigen from the urinary isolate or a standard homologous antigen a pool of 8 common *E coli* O antigens (8 antigen pool) or an O antigen pool derived from 68 *E coli* isolated from urinary specimens in the laboratory during several years (68 antigen pool)* The latter pool included the aforementioned 8 O antigens On the basis of experiments analyzing the lowest concentration of the individual antigens permitting detection of the specific O antibodies in rabbit immune sera without significant titre loss it was regarded appropriate to mix equal parts of a stock solution (O D 0 73) of each O antigen although this resulted in a 1/68 dilution of the individual antigens

Treatment of serum with fresh 2 mercaptoethanol (ME) was performed to differentiate reduction sensitive from reduction resistant antibodies (9) The relation of these two types of antibodies to molecular size was tested Twenty five sera from children with UTI were

O groups 1 2 4 6 7 8 18 and 75

* O groups 1–15 17–23 25–26 28 33–34 36 42 46 48 51 55 59–60 65 68–69 71 75–78 80–83 85–86 88 91 98–99 101 110–111 11? ab 112 ac 115 117–120 125 1 8 133 140 145

fraction ited by Sephadex G 200 gel filtration (9) and the 19 S and 7 S fractions were tested in indirect hemagglutination These patients were not otherwise included in the present study

Statistical methods

The chi square test was used for the statistical calculations (27) Stepwise discriminant analysis was performed on an IBM 360 computer using the MBD 07M program (7)

RESULTS

Sensitivity of anti O antibody to reduction with ME

The O antibody activity in the 19 S gel filtration fractions of 25 patient sera was highly sensitive to reduction with ME a mean decrease from 7 8 to 1 4 titre steps (−log2) was noted The 7 S fractions of these sera were largely resistant to this treatment with a mean titre decrease from 6 2 to 5 1 Titres obtained with ME reduced sera were therefore considered to represent mainly the 7 S antibody levels

Control group

Titration of sera from healthy individuals was performed using the two antigen pools Since no urinary isolate existed for the healthy individuals the results obtained with the 8 antigen pool were chosen for comparison with patient titres obtained using the homologous antigen The titre of the 95th percentiles for age with the 8 antigen pool are indicated for nonreduced antibody in Fig 1 *a* and for ME resistant antibody in Fig 1 *b* Titres above this level are referred to as increased

Titration with homologous antigen

Fig 1 *a* and *b* shows the highest O agglutinin titres obtained with unreduced and reduced sera from patients with pyelonephritis and cystitis, and their relation to the titres in the controls Only two of the 40 patients (5%) with cystitis had increased antibody titres in unreduced serum In contrast 48 of the 54 patients (89%) with pyelonephritis

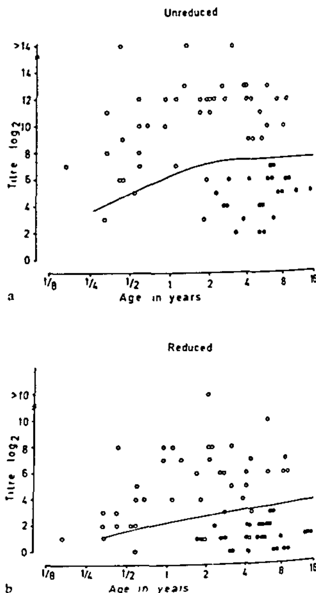


Fig 1 Maximal antibody titres against the O antigen from the urinary isolate registered in cases of primary symptomatic UTI (*a*) Titres of unreduced sera (*b*) Titres of ME reduced sera The inserted curve indicates the 95th percentile of titres of 643 healthy controls in relation to age O cases of acute pyelonephritis ● cases of acute cystitis

(Table 1) demonstrated increased titres a highly significant difference ($p<0\ 001$) With ME treated sera none of the patients with cystitis but 44 of the patients with pyelonephritis (81%) had elevated titres

Sera from the pyelonephritis patients showed a predominance of 19 S antibodies Thus in 50 of the 54 patients ME reduction of the serum lowered the titre by three or more steps i e approximately 1/8 or less of

Table 1 *Number of patients with elevated antibody titres by (A) comparison with the 95th per centile of the titres in the normal population in relation to age and by (B) discriminant analysis on a computer*

– = serum unreduced + = serum reduced

Antigen: Homologous –	Homologous +	8 antigen pool –	8 antigen pool +	68-antigen pool –	68-antigen pool +	A 95th percentile: Pyelonephritis ($n=54$)	A 95th percentile: Cystitis ($n=40$)	B Discriminant analysis: Pyelonephritis ($n=54$)	B Discriminant analysis: Cystitis ($n=40$)
×						48	2	50	1
	×					44	0	47	1
		×				39	3	46	1
			×			25	0	42	2
				×		33	2	43	
					×	10	0	38	4
×	×					50	2	50	1
		×	×			41	3	45	0
				×	×	37	2	44	3
		×	×	×	×	45	4	46	1
×	×	×	×	×	×	3	5	50	0

the titre was caused by 7 S antibodies In three cases the reduction was two titre steps and in one serum only one step

The maximal titre of unreduced sera was attained by a mean of 8 days while the maximal titre of reduced sera was found 17 days after onset of the symptoms The proportion of increased titres in relation to sampling is shown in Fig 2

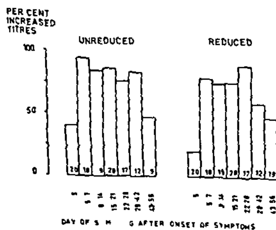


Fig 2 Per cent increased titres in sera from pyelonephritis patients in relation to onset of symptoms The number of sera obtained during each period is indicated by the figures at the bottom of the columns

Titration with the antigen pools

Using the antigen pools and serum unreduced three of the 40 patients with cystitis (8%) had elevated titres with the 8 pool and two with the 68 pool one of them having increased titres to both pools In the patients with pyelonephritis unreduced sera gave elevated titres with the 8 antigen pool in 39 (72%) and with the 68 antigen pool in 33 cases (61%) (Table 1) The difference between the results for the patients with cystitis and those with pyelonephritis is highly significant ($p<0.001$) whereas the difference between the results obtained with the two pools using sera from patients with pyelonephritis is not ($p>0.05$) Of the 37 patients with pyelonephritis caused by *E coli* of O groups included in the 8 antigen pool 36 (97%) had increased titres of unreduced serum While the titres against the 8 antigen pool were similar to those against the homologous single antigen the 68 antigen pool gave considerably lower titres and only rarely more than 2 to 3 steps above the normal level With respect to reduction resistant antibodies the number of sera with elevated titres detected using the 8 antigen pool was

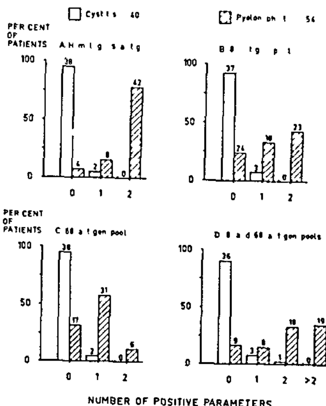


Fig 3 Discrimination between patients with cystitis and pyelonephritis provided by indirect hemagglutination using the 95th percentile of titres of age matched healthy controls as reference The two parameters used were maximal titres of (*a*) unreduced and (*b*) reduced sera The distribution of patients with 0 1 and 2 positive parameters is given in per cent The number of patients in the groups is indicated by the figures above each column

lower than with the homologous antigen ($p<0\ 01$) and even lower with the 68 antigen pool (Table 1) The difference between the pools was also significant ($p<0\ 01$)

Combined use of unreduced and ME reduced serum antibody

Fig 3 *a–d* shows how the combined use of antibody titres in unreduced and reduced sera adds to the discrimination between pyelonephritis and cystitis Thus for instance with the homologous O antigen both of these titres were increased only in sera from pyelonephritis patients (78%)

Sequential titre changes

The data were also analysed for significant titre alterations (>2 titre steps) between sequential serum samples from the same individual Such changes were found only in sera from one patient with cystitis with each of the antigen preparations which contrasts to the pyelonephritis group in which 42 (78%) changed significantly with homologous antigen 32 (59%) with the 8 and 19 (35%) with the 68 antigen pool With ME-reduced sera no cystitis patient showed significant changes and the figures for the pyelonephritis group were 37 (69%) 21 (39%) and 4 (7%) respectively Using homologous antigen (45 (83%) of the pyelonephritis patients had changes of titres in unreduced and/or ME reduced serum

The correlation between the clinical diagnosis and the result of the serological tests including evaluation of maximal titre of unreduced and reduced sera together with their sequential changes is shown in Fig 4 With this combination of four tests at least one parameter was found elevated (positive) in 50 (93%) with the homologous antigen and in 41 (76%) and 38 (70%) with the 8 and 68 antigen pools of the 54 pyelonephritis patients The latter pool discovered 4 patients not revealed by the 8 antigen pool and thus the use of both pools increased the figure to 83% This increase however was not statistically significant ($p>0\ 05$)

With the homologous antigen two or more parameters with increased values were seen only in patients with pyelonephritis (89% of this group) Also with the 8 antigen pool a very good discrimination was obtained significantly better than with the 68 antigen pool

Discriminant analysis

Using the maximal titres of unreduced and ME reduced sera together with the age of the patients as variables stepwise discriminant analysis was performed Table 1 shows that the discrimination obtained in this way was somewhat better than when the 95 percentile of titres in sera from healthy controls was used as reference to identify increased titres Formulas derived on the computer were used

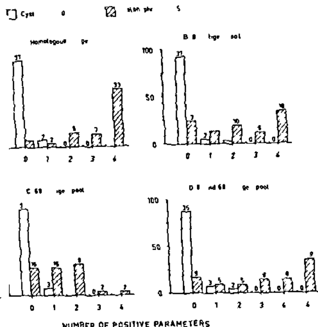
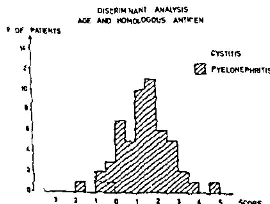


Fig 4 Discrimination between patients with cystitis and pyelonephritis provided by indirect hemagglutination using the 95th percentile of titres of age matched healthy controls as reference The four parameters analysed were (*a*) maximal titres of unreduced and (*b*) reduced sera (*c*) significant change in titres of unreduced and (*d*) reduced sera The distribution of patients with from 0 to 4 positive parameters is given in per cent The number of patients in the groups is indicated by the figures above each column

to calculate the score for each patient and examplifying results are given as histograms in Fig 5 *a* and *b* An excellent discrimination between pyelonephritis and cystitis was obtained from such scores using the homologous antigen Also with the 8 antigen pool a very good differentiation was found which did not increase further when data of the 68 antigen pool were added

DISCUSSION

Titration of serum antibodies using O antigen prepared from the bacteria isolated in the urine or a standard homologous antigen showed elevated antibody levels in 89% of patients with a first known attack of symptomatic pyelonephritis Using the same procedure sera from girls with a first attack of



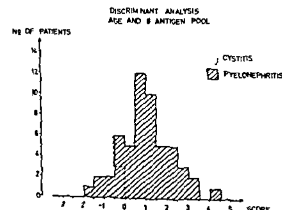


Fig 5 Discrimination of patients with cystitis and pyelonephritis provided by indirect hemagglutination with (*a*) homologous antigen and (*b*) the 8 antigen pool using a computer for stepwise discriminant analysis The scores were calculated from the formulas

(*a*) 0 32×(−log2 nonreduced titre)+0 01×(−log2 reduced titre)−[illegible]×log 10 age years−1 54

(*b*) 0 24×(−log2 nonreduced titre)+0 21×(−log2 reduced titre)−3 49×log 10 age years−1 16

symptomatic cystitis in only 5% were found to be increased above the 95 percentile of antibody titres of age matched controls. A more detailed evaluation including estimations of antibody classes and sequential titre changes permitted recognition of pyelonephritis with only a minimal risk of including patients with cystitis. Thus two or more of the employed parameters, increase of reduction sensitive and resistant antibodies and titre changes in sequential samples, were present only in patients with pyelonephritis (89%). To obtain this discrimination, however, adequate timing of serum sampling is required, i.e. optimally around two to three weeks after onset of symptoms (cf. Fig. 2). It is also obvious that care should be taken to avoid denaturation of antibodies by improper handling (3), that the antigen is prepared from the pertinent urinary pathogen and that the patient has no immunologic deficiency.

An interesting observation was that so many of the patients with pyelonephritis responded with formation of ME resistant 7 S O antibodies, evident both as elevated titres of such antibodies and as titre changes following the infection. The 7 S antibody formation in primary immune responses to lipopolysaccharides therefore seems to differ considerably between mammal species, being substantial in humans and rabbits (13) but hardly detectable in mice (1, 6). Diagnostically in this material consisting of girls with their first UTI, the registration of ME resistant antibodies did not markedly improve the already good correlation between clinical diagnosis and antibody which was obtained by studies of total agglutinin level. However, determination of both the non-reduced and the ME resistant agglutinin titres gave more pronounced separation between pyelonephritis and cystitis, as only patients with renal engagement had elevated titres of both types of agglutinins (78%). When titre changes were studied as well, additional discrimination was found (89%).

Determination of ME resistant O antibody titres may be of additional diagnostic value in recurrent UTI, since preliminary data indicate that precipitating IgG antibodies are found more often after recurrent than after primary pyelonephritis (10).

Confirming the work of Andersen (2) it was found that the antigen from the urinary isolate could be replaced by a pool of the 8 most common O antigens. When the urinary pathogen was of an O group represented in this pool, elevated maximal titres were noted in all but one case and the titre levels were similar to these obtained with antigen from the homologous strain. In the present material 69% of the patients with pyelonephritis and 63% of patients with cystitis had infections caused by bacteria of these O groups. By including sequential determinations and ME reduction the agreement between clinical diagnosis and titration using the 8 antigen pool was 76% irrespective of the O group of the urinary isolate. Thus a few patients with infections of O groups outside this pool were also recognized. This was probably due to cross reactions between the O antigens of the urinary isolates and the O antigens included in the pool. The low frequency of increased titres in the patients with cystitis was not changed by the use of this antigen pool.

A larger pool of 68 O antigens was also tested. With this pool the overall frequency of elevated titres was 61% when unreduced serum was studied and 70% when 7 S antibodies and sequential titre changes were also considered. Thus, this large pool was no better than the 8 antigen pool, instead it gave a lower number of elevated titres than the smaller pool. Adding the results from the use of the two pools increased the number of elevated titres insignificantly from 76% to 83%. A confirmatory serological diagnosis is thus obtained in only a few additional cases of primary acute pyelonephritis by the combined use of the two pools. In our opinion this increase is too small to justify the considerable

extra effort involved in routine use of both pools in the serological diagnosis of primary symptomatic UTI It may be discussed if the use of the large pool would be of greater value for studies of recurrent UTI where the frequency of *E coli* O groups outside the 8 antigen pool seems to be higher (un published) The low capacity of the 68 antigen pool to detect elevated 7 S antibody titres which may be of special diagnostic value in these infections (10) constitutes a limitation however which might motivate the effort of preparing antigen from the uri nary isolate in these cases

This study proves the efficiency of anti body determination in localization of acute UTI in a clinically well characterized patient material thereby confirming previous in vestigations of correlation between antibody titre and level diagnosis from clinical findings (19 23 25 27) or from enumeration of bacteria in ureteric catheter urine (12 21) In the present study the diagnosis of cystitis or pyelonephritis was based on clinical and laboratory findings with high fever together with transiently decreased renal concentrat ing capacity and/or elevated sedimentation rate as indications of renal engagement In another study comparing different methods for localization of symptomatic UTI these laboratory tests were found to correlate well with the clinical diagnosis in fact better than the bladder washout technique (16)

The present study confirms the validity of using serum antibody determinations as an aid in level diagnosis of childhood UTI Such determinations may be a valuable help in situations where other criteria do not easily permit a level diagnosis or where symptoms and signs indicate a renal infection but the urine findings are normal (4) Further studies will have to show whether antibody determinations are similarly useful in chil dren with recurrent and asymptomatic UTI

However even more important than to establish the level of infection in a single attack of UTI would be to have a simple method permitting evaluation of the risk of attracting repeated renal infections and progressive renal damage As yet no method for detection of the patients at risk has been described and further studies of the antibody response seem motivated especially concern ing antibodies of the serum IgG type and of urine antibodies (14 15)

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The discriminant analysis was performed by Hans Wedel and Bjorn Pettersson Department of Mathematics Chalmers Institute of Technology and University of Goteborg

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REFERENCES

1 Ahlstedt S Holmgren J & Hanson L Å The primary and secondary antibody response to *Escherichia coli* 06 lipopolysaccharide analysed at the humoral and cellular level Amount and avidity of the antibodies in relation to protective capacity *Immunology* 24 191 1973
2 Andersen H J Studies of urinary tract infections in infancy and childhood IX Determination of *E coli* antibodies by a polyvalent antigen *Acta Paediatr Scand* 56 637 1967
3 — Clinical studies on the antibody response to *E coli* O antigens in infants and children with urinary tract infection using a passive haemagglu tination technique *Acta Paediatr Scand* Suppl *180* 1968 pp 8
4 Andersen H J Bergstrom T Lincoln K Ørskov F Ørskov I & Winberg J Studies of urinary tract infections in infancy and childhood VI Deter mination of coli antibody titres in the diagnosis of acute urinary tract infections lacking the usual urinary findings *J Pediatr 67* 1080 1965
5 Andersen H J Hanson L Å Lincoln K Ørskov F & Winberg J Studies of urinary tract infections in infancy and childhood IV Relation of the coli antibody titre to clinical picture and to serological type of the infecting *Escherichia coli* in acute uncomplicated urinary tract infec tions *Acta Paediatr Scand 54* 247 1965
6 Britton S & Moller G Regulation of antibody synthesis against *Escherichia coli* endotoxin Suppressive effe t of endogenously produced and passively transf rred antibodies *J Immunol 100* 13 6 1968
7 Dixon W J *Biomedical computer programs* University of California Press Los Angeles 1971

symptomatic cystitis in only 5% were found to be increased above the 95 percentile of antibody titres of age matched controls A more detailed evaluation including estimations of antibody classes and sequential titre changes permitted recognition of pyelonephritis with only a minimal risk of including patients with cystitis Thus two or more of the employed parameters increase of reduction sensitive and resistant antibodies and titre changes in sequential samples were present only in patients with pyelonephritis (89%) To obtain this discrimination however adequate timing of serum sampling is required i e optimally around two to three weeks after onset of symptoms (cf Fig 2) It is also obvious that care should be taken to avoid denaturation of antibodies by improper handling (3) that the antigen is prepared from the pertinent urinary pathogen and that the patient has no immunologic deficiency

An interesting observation was that so many of the patients with pyelonephritis responded with formation of ME resistant 7 S O antibodies, evident both as elevated titres of such antibodies and as titre changes following the infection The 7 S antibody formation in primary immune responses to lipopolysaccharides therefore seems to differ considerably between mammal species being substantial in humans and rabbits (13) but hardly detectable in mice (1 6) Diagnostically in this material consisting of girls with their first UTI the registration of ME resistant antibodies did not markedly improve the already good correlation between clinical diagnosis and antibody which was obtained by studies of total agglutinin level However, determination of both the non reduced and the ME resistant agglutinin titres gave more pronounced separation between pyelonephritis and cystitis as only patients with renal engagement had elevated titres of both types of agglutinins (78%) When titre changes were studied as well additional discrimination was found (89%)

Determination of ME resistant O antibody titres may be of additional diagnostic value in recurrent UTI since preliminary data indicate that precipitating IgG antibodies are found more often after recurrent than after primary pyelonephritis (10)

Confirming the work of Andersen (2) it was found that the antigen from the urinary isolate could be replaced by a pool of the 8 most common O antigens When the urinary pathogen was of an O group represented in this pool elevated maximal titres were noted in all but one case and the titre levels were similar to these obtained with antigen from the homologous strain In the present material 69% of the patients with pyelonephritis and 63% of patients with cystitis had infections caused by bacteria of these O groups By including sequential determinations and ME reduction the agreement between clinical diagnosis and titration using the 8 antigen pool was 76% irrespective of the O group of the urinary isolate Thus a few patients with infections of O groups outside this pool were also recognized This was probably due to cross reactions between the O antigens of the urinary isolates and the O antigens included in the pool The low frequency of increased titres in the patients with cystitis was not changed by the use of this antigen pool

A larger pool of 68 O antigens was also tested With this pool the overall frequency of elevated titres was 61% when unreduced serum was studied and 70% when 7 S antibodies and sequential titre changes were also considered Thus this large pool was no better than the 8 antigen pool instead it gave a lower number of elevated titres than the smaller pool Adding the results from the use of the two pools increased the number of elevated titres insignificantly from 76% to 83% A confirmatory serological diagnosis is thus obtained in only a few additional cases of primary acute pyelonephritis by the combined use of the two pools In our opinion this increase is too small to justify the considerable

extra effort involved in routine use of both pools in the serological diagnosis of primary symptomatic UTI It may be discussed if the use of the large pool would be of greater value for studies of recurrent UTI where the frequency of *E coli* O groups outside the 8 antigen pool seems to be higher (un published) The low capacity of the 68 antigen pool to detect elevated 7 S antibody titres which may be of special diagnostic value in these infections (10) constitutes a limitation however which might motivate the effort of preparing antigen from the uri nary isolate in these cases

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However even more important than to establish the level of infection in a single attack of UTI would be to have a simple method permitting evaluation of the risk of attracting repeated renal infections and progressive renal damage As yet no method for detection of the patients at risk has been described and further studies of the antibody response seem motivated especially concern ing antibodies of the serum IgG type and of urine antibodies (14 15)

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REFERENCES

1 Ahlstedt S Holmgren J & Hanson L Å The primary and secondary antibody response to *Escherichia coli* 06 lipopolysaccharide analysed at the humoral and cellular level Amount and avidity of the antibodies in relation to protective capacity *Immunology 24* 191 1973
2 Andersen H J Studies of urinary tract infections in infancy and childhood IX Determination of *E coli* antibodies by a polyvalent antigen *Acta Paediatr Scand 56* 637 1967
3 — Clinical studies on the antibody response to *E coli* O-antigens in infants and children with urinary tract infection u ing a pa sive haemagglu tination technique *Acta Paed atr Scand* Suppl *180* 1968 pp 8
4 Andersen H J Bergstrom T Lincoln K Ørskov F Ørskov I & Winberg J Studies of urinary tract infections in infancy and childhood VI Deter mination of coli antibody titres in the diagnosis of acute urinary tract infections lacking the usual urinary findings *J Pediatr 67* 1080 1965
5 Andersen H J Hanson L Å Lincoln K Ørskov F & Winberg J Studies of urinary tract infections in infancy and childhood IV Relation of the coli antibody titre to clinical picture and to serological type of the infecting *Escherichia* coli in acute uncomplicated urinary tract infec tions *Acta Paediatr Scand 54* 247 1965
6 Britton S & Möller G Regulation of antibody synthesis against *Escherichia coli* endotoxin Suppressive effect of endogenously produced and passively transferred antibodies *J Immunol 100* 13 6 1968
7 Dixon W J *Biomed cal computer programs* University of California Press Los Angeles 1971

8 Fairley K F Grounds A D Carson N E Laird E C Gutch R C McCallum P H G Leighton P Sleeman R I & O Keefe C M Site of infection in acute urinary tract infection in general practice *Lancet II* 615 1971
9 Hanson L Å Holmgren J Jodal U & Kaijser B Characterization of precipitating antibodies to *E coli* O antigen in infants and children with acute pyelonephritis *Clin Exp Immunol 8* 573 1971
10 Hanson L Å Holmgren J Jodal U & Winberg J Precipitating antibodies to *E coli* O antigens a suggested difference in the antibody response of infants and children with first and recurrent attacks of pyelonephritis *Acta Paediatr Scand 58* 506 1969
11 Hanson L Å & Winberg J Demonstration of antibodies of different immunoglobulin types to the O antigen of the infecting *E coli* strain in infants and children with pyelonephritis *Nature 212* 1495 1966
12 Hewstone A S & Whitaker J The correlation of ureteric urine bacteriology and homologous antibody titer in children with urinary infection *J Pediatr 74* 540 1969
13 Holmgren J The antibody response in rabbits to *E coli* O antigen *Int Arch Allergy 37* 546 1970
14 Holmgren J Hanson L Å Jodal U Kaijser B & Winberg J Studies of the antibody response to *E coli* in children with urinary tract infection and its possible protective implications as analysed in experimental animals *In* H Losse & M Kienitz (eds) *Pyelonephritis* Band III Thieme Verlag Stuttgart 1972 p 126
15 Jodal U Ahlstedt S Carlsson B Hanson L Å Lindberg U & Sohl A Local antibodies in childhood urinary tract infection A preliminary study *Int Arch Allergy 47* 537 1974
16 Jodal U Lindberg U & Lincoln K Level diagnosis of symptomatic urinary tract infections in childhood *Acta Paediatr Scand* in press
17 Lincoln K Lidin Janson G & Winberg J Resistant urinary infection resulting from changes in resistance pattern of faecal flora induced by sulphonamide and hospital environment *Brit Med J III* 305 1970
18 Mond N C Percival A Williams J D & Brumfitt W Presentation diagnosis and treatment of urinary tract infections in general practice *Lancet I* 514 1965
19 Percival A Brumfitt W & de Louvois J Serum antibody levels as an indication of clinically inapparent pyelonephritis *Lancet II* 1027 1964
20 Pike R M Schulze M L & Chandler C H Agglutinating and precipitating capacity of rabbit anti *Salmonella typhosa* γG and γM antibodies during prolonged immunization *J Bact 92* 880 1966
21 Reeves D S & Brumfitt W Localization of urinary tract infection A comparative study of methods *In* F O Grady & W Brumfitt (eds) *Urinary tract infection* Oxford University Press London 1968 p 53
22 *Scientific tables* Geigy SA Basel 1962 6th ed p 165
23 Vosti K L Monto A S & Rantz L A Host parasite interaction in patients with infections due to *Escherichia coli* II Serologic response of the host *J Lab Clin Med 66* 613 1965
24 Vosti K L & Remington J S Host-parasite interaction in patients with infections due to *Escherichia coli* III Physicochemical characterization of O specific antibodies in serum and urine *J Lab Clin Med 72* 71 1968
25 Williamson J Brainerd H Scaparone M & Chueh S P Antibacterial antibodies in coliform urinary tract infections *Arch Int Med 114* [illegible] 1964
26 Winberg J Determination of renal concentration capacity in infants and children without renal disease *Acta Paediatr Scand 48* 318 1959
27 Winberg J Andersen H J Hanson L Å & Lincoln K Studies of urinary tract infections in infancy and childhood I Antibody response in different types of urinary tract infections caused by coliform bacteria *Brit Med J II* 524 1963

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TREATMENT OF RECURRENT URINARY TRACT INFECTION IN CHILDREN

II Compliance of Parents and Children with Antibiotic Therapy Regimen

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ABSTRACT Daschner F and Marget W (Division for Antimicrobial Therapy Children s Hospital University of Munich BRD) Treatment of recurrent urinary tract infection in children Acta Paediatr Scand 64 105 1975.—Since none of the studies of long term management of recurrent urinary tract infections considered the possibility of patients not taking medication compliance with long term antibiotic therapy was tested by urine check in 93 children with recurrent urinary tract infection Only 30 children (32 2%) took the prescribed drugs at regular intervals 27 children (19 1%) did not take the antibiotics at all and 36 patients took the drugs irregularly skipping one or two doses a day The difference in infection rate between regular takers (3 9/year) and non takers (7 2/year) and irregular takers (4.8/year) and non takers was statistically highly significant Forgetfulness and negligence of the parents was found to be the main reason for not giving the child the medication Treatment studies which show the advantage of one regimen over another or which try to define optimal duration of therapy should take particular care in evaluation compliance

KEY WORDS Urinary tract infection treatment long term compliance antibiotic therapy

Several investigations on children with recurrent urinary tract infections (UTI) suggested that long term therapy could reduce the frequency of recurrences better than short term treatment (13 14 16) Others reported that the frequency of recurrence in girls with first time infections was not diminished by extending the sulfonamide administration from 10 days to 2 months (3) Although the poor rate of cooperation of mothers and children especially in long term medical regimens is well documented (1) none of the studies of long or short term management of recurrent UTI considered the possibility of children not taking medication The judgement of success or failure of any therapy however depends essentially on proper intake of the prescribed drug Therefore this investigation was planned to study the compliance of mothers and children with antibiotic therapy regimens in recurrent UTI in relation to the rate of relapse An analysis was also made as to why children did not receive the prescribed medication

MATERIAL AND METHODS

105 patients (aged from 1½ to 13½ years) with recurrent UTI on long-term therapy (for an average of 24 3 months) participated in this study 15 patients had obstructive lesions of the urinary tract all children had at least 3 infections of the urinary tract within 1 year prior to the study period and at least 3 infections prior to the follow-up in our urologic clinic All patients were seen in rotation by 4 different physicians

Table 1 *Sensitivity of assay technique*

Antibiotics	Minimum concentration of antibiotic producing zone of inhibition (µg/ml)	
	E coli	*Bact subtilis*
Nitrofurantoin	10	20
Nalidixic acid	10	10
Cephalexin	20	10
Gentamicin	1	1
Ampicillin	3	2
Chloramphenicol	1	10
Sulfamethoxazole	20	-

Non significant bacteriuria in pure culture in mid stream urine was considered as a sign of infection rather than contamination when two of the following criteria were concurrently met (6)

(*a*) pyuria (defined as more than 50 leukocytes/µl uncentrifuged fresh urine)

(*b*) at least two clinical symptoms suggesting urinary tract infection (e g frequency burning temperature loin pain)

(*c*) four fold increase of homologous antibody titre against the infecting strain determined with indirect hemagglutination (12 18)

(*d*) non significant bacteriuria following or preceding a significant bacterial count of the same bacterial species within 3 weeks

About 30% of all relapses in this study group were caused by non significant bacteriuria mostly in counts between 10^4–10^5 bacteria/ml urine All children attended the special urologic service and were followed up for 1–5 years prior to the study period At each clinic visit (average 22 3 visits per patient) patients were instructed to take the prescribed medication at regular intervals following breakfast lunch and dinner In order to check the compliance of parents and children a letter with six strips of filter paper was sent to the parents with instructions on how to participate in a now renal function test Parents had to dip a strip into the mid stream urine three times daily at the expected time of maximum antibiotic excretion (first morning urine before lunch and before bed time) This had to be done twice weekly for 1 week (with a 2 day interval) The filter paper strips (Schleicher & Schuller Nr 2668 Dassel BRD) had an area of 2×3 cm soaked with paraffin which prevented unequal amounts of urine on the strips After use the strips were dried in the dark overnight by the parents and sent by mail to our laboratory in a plastic envelope Laboratory studies revealed that even drying in sunlight or storage for up to 1 week did not decrease the antibiotic concentration of the discs by inactivation or degradation Eight discs (diameter 0 5 cm) were punched out from the area dipped in urine Four discs each were placed in cloverleaf form on plates containing 6 ml agar (D S T Agar Oxoid) and inoculated with 0 1 ml of a 1 10^4 dilution (*E coli*) or 1 10^2 dilution (*Bact subtilis*) of a 5 hour culture of the test organisms in brain heart infusion broth (Difco) Any inhibition zone between or around the four discs indicated antibiotic excretion in the urine

Sensitivity of our assay method is indicated in Table 1 The accuracy of the method was tested in 10 hospitalized children on a regular diet and normal fluid intake who received 5 mg/kg body weight of nitrofurantoin in three divided doses Antibiotic activity could be demonstrated in all children by the described technique In addition 241 patients not receiving chemotherapy were tested in order to check the intake of antibiotics other than those used for treatment of UTI

RESULTS

200 (83%) of the 241 patients of children not receiving chemotherapy and 98 (93 3%) of the 105 children on long term chemotherapy participated in our study An average of 14% of all parents did not appear to be interested in a renal function test of their children Three of the 200 children not being treated for urinary tract infection were taking antibiotics for treatment of upper respiratory tract infections Parents of 5 children failed to send all paper strips so that complete urine checks could be done on 93 children on long term chemo therapy Of these 93 children 66 were being treated with nitrofurantoin (4 5 mg/kg body weight daily) 15 with ampicillin (25–75 mg/kg body weight) 11 were receiving sulfonamides (80–120 mg/kg body weight daily) and 1 child was receiving cephalexin (50 mg/kg body weight) In 5 children the follow up could not be completed Results of the test are given in Table 2 Only 30 children took the prescribed drugs at regular intervals 27 children did not take the antibiotics at all and 36 children took the drugs irregularly skipping one or two doses a day Statistically significant differences in infection rates including non significant bacteriuria as described previously (6) were found between regular takers and non takers of the medication No difference in compliance on ampicillin nitrofurantoin or sulfonamides was found

Table 2 *Compliance of children with recurrent urinary tract infection with long term antibiotic therapy according to urine check*

	No of patients	%	Infection rate/Year		Statistical significance p value
Children under long term therapy	93	100			
Regular takers	30	32 2	3 9	$p<0\ 20$ (3 9 vs 4 8)	$p<0\ 001$
Irregular takers	36	38 7	4 8	$p<0\ 01$ (4 8 vs 7 2)	
Non takers	27	29 1	7 2		

An important finding was that even irregular ingestion of antibiotics reduced the incidence of infections The infection rate in the group of irregular takers was significantly lower than in the non taker group Personal interviews after the test with all parents of non takers and most parents of irregular takers confirmed our experimental results The reasons for not taking the prescribed medications are indicated in Table 3

DISCUSSION

When we related the patients chemotherapy to the incidence of bacteriuria we often found two types of cases in long term follow up One was infection after cessation of long term therapy which indicated successful prevention of relapse or reinfection during therapy and the other was relapse or reinfection despite long term therapy The latter could not be explained on the basis of resistant organisms alone This prompted the present study to control whether or not the children on long term chemotherapy took the prescribed antibiotics Counting of remaining tablets after a complete course of therapy or personal interviews concerning regular intake of the medication have not been found to be reliable methods for checking compliance (2 7) Assay of antibacterial activity in urine however has been proved an adequate check that the drug has been taken (9) In the literature the incidence of non compliance with regimens of oral antibiotics varies between 1% and 82% (2 5 10) Jackson et al found only 1% non-compliers after controlling a rural population treated for streptococcal pharyngitis whereas 82% of all patients had stopped taking penicillin by the ninth day despite instructions for a full 10-day course in Bergmans study (2) Other studies have reported patient non compliance in rheumatic fever (8) rheumatoid arthritis (11) peptic ulcer (15) tuberculosis (4) and in the use of tranquillizers (5) As far as could be determined this is the first study of compliance in children with recurrent UTI As in other patients with chronic mostly asymptomatic infections (4 8 11) this study reveals that this group of patients is a high risk one for non compliance with long term chemotherapy The main reason that children did not receive medication was forgetfulness or negligence on the part of the parents Whether better

Table 3 *Parents reasons resulting from personal interviews as to why they did not give the medication*

Parent s reasons	No of parents
I have forgotten to give the medication	12
My child did not have *symptoms anymore*	4
My child threw the tablets away	4
My private physician discontinued the medication	3
The tablets do not help	2
The tablets make my child sick	1
When my child is not *feeling well I* do not give him the medication	1
Total	27

patient–physician contact or use of behavioural models would improve compliance of mothers with pediatric medical regimens —as suggested by Becker et al (1)—remains to be investigated All of the children's parents were carefully instructed by interviews and also by information forms prior to the study period that outlined the incidence danger prognoses follow up and reasons for long term chemotherapy of recurrent UTI Every parent knew each of the four physicians on the special urologic service for months or even years

Several conclusions can be drawn from this study Firstly treatment studies which show the advantages of one regimen over another or which try to define optimal duration of therapy must take particular care in evaluation compliance Secondly many children in this study did not receive the prescribed medication and this may well occur in other comparable groups Thirdly long term chemotherapy and even irregular intake of antibiotics significantly reduces the incidence of relapse or reinfection and finally forgetfulness and negligence on the part of the mothers probably worsens the prognosis of children with recurrent UTI

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REFERENCES

1 Becker M H Drachmann R H & Kirscht J P Predicting mothers compliance with pediatric medical regimens *J Pediatr 81* 843 1972
2 Bergmann A B & Werner R J Failure of children to receive penicillin by mouth *N Engl J Med 268* 1334 1963
3 Bergström R Lincoln K Redin B & Winberg J Studies of urinary tract infections in infancy and childhood X Short or long term treatment in girls with first or second time urinary tract infections uncomplicated by obstructive urological abnormalities *Acta Paediatr Scand 57* 186 1968
4 Berry D Ross A Huempfener H & Deuschle K Self medication behaviour as measured by urine chemical test in domiciliary tuberculous patients *Am Rev Resp Dis 86* 1 196?
5 Charney E Dynus R Eldredge D McWhinney J McNabb N Scheiner A Sumpter E & Iker H How well do patients take oral penicillin? A collaborative study in private practice Pediatrics 40 188 1967
6 Daschner F Grau M Kölling A & Marget W Die Bedeutung nichtsignifikanter Keimzahlen bei zuverlässig kontrollierter Therapie chronischer Harnweginfektionen *Monatschr Kinderheilkd 121* 486 1973
7 Gordis L Markowitz M & Lilienfeld A M The inaccuracy in using interviews to estimate patients reliability in taking medications at home *Med Care 7* 49 1969
8 — Studies in the epidemiology and preventability of rheumatic fever IV A quantitative determination of compliance in children on oral penicillin prophylaxis *Pediatrics 43* 173 1969
9 Harvey K J Antibacterial activity in urine *Med J Aust 1* 1072 1972
10 Jackson H Cooper J Nellinger W & Olsen A Streptococcal pharyngitis in rural practice *JAMA 197* 385 1966
11 Joyce C R B Patient cooperation and the sensitivity of clinical trials *J Chronic Dis 15* 1025 196?
12 Neter E Bacterial hemagglutination and hemolysis *Bact Rev 20* 166 1956
13 Normand J C S & Smellie J M Prolonged maintenance chemotherapy in the management of urinary infection in children *Br Med J 1* 1023 1965
14 Pryles Ch V Wherrett B A & McCarthy J M Urinary tract infection in infants and children Long term prospective study interim report on results of six weeks chemotherapy *Am J Dis Child 108* 1 1964
15 Roth H & Berger D G Studies on patient cooperation in ulcer treatment *Gastroenterology 38* 630 1960
16 Stansfeld J M & Webb J K G Plea for longer treatment of chronic pyelonephritis in children *Br Med J 1* 616 1954
17 Wilcox D R C Gillan R & Hare E H Do psychiatric outpatients take their drugs? *Br Med J II* 790 1965
18 Winberg J Andersen H J Hanson L A & Lincoln K Studies of urinary tract infections in infancy and childhood I Antibody response in different types of urinary tract infections caused by coliform bacteria *Br Med J II* 506 1971

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GASTRIC ASPIRATE ANALYSIS IN THE NEWBORN

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ABSTRACT Graham Pole J and McAllister T A (Departments of Child Health and Bacteriology Royal Hospital for Sick Children and Queen Mother s Hospital Yorkhill Glasgow Scotland) Gastric aspirate analysis in the newborn Acta Paediatr Scand 64 109 1975 —The value of gastric aspirate analysis in screening for perinatal infection is disputed We have studied the cellularity and bacterial content of 231 aspirates from 105 normal infected and asphyxiated infants with the following conclusions (1) A positive culture has no pathological significance (2) Aspirate cellularity does not correlate with bacterial culture results (3) An excess of polymorphonuclear leucocytes (PMNL) in aspi rates collected at birth is a pathological but non specific stress response (4) After birth there is a physiological rise and fall in the aspirate PMNL content parallel to that in the peripheral blood Aspirate cellularity and bacterial culture are therefore of limited value in the diagnosis of neonatal pneumonia

KEY WORDS Neonatal gastric aspirates polymorphonuclear leucocytes pneumonia asphyxia

The polymorphonuclear leucocyte (PMNL) content of gastric aspirates is used to diag nose neonatal pneumonia (6 8) but may well lack specificity (4) We have evaluated it as a diagnostic test by counting aspirate PMNL in infected asphyxiated and normal infants

PATIENTS AND METHODS

231 aspirates were anlysed from 105 unselected infants between birth and the fourth day of life These comprised single aspirates from 72 and serial aspirates from 35 collected with a Jaques 14 F G catheter into a sterile container either at birth or at least 4 hours after a feed Because aspirate consistency varied greatly it was not valid to make a total cell count in a known volume Two 100-cell counts were therefore made from methylene blue and gram-stained smears and the PMNL percentage calculated Aspirates were diluted with 1 100 and 1 1000 sterile saline incubated at 37°C in MacConkey blood and Sabouraud agars and colonies counted with a Gallenkamp counter 35 healthy infants (23 mature 12 premature) had serial aspirate and capillary blood samples collected for differential leucocyte counts at 0–1 10–12 22–26 46–50 and 94–98 hours after birth This was in order to follow concomitant physiological changes in blood and aspirate PMNL counts These infants had normal cord and placenta histology and cultures from nose throat groin and rectum as well as gastric aspi rates contained no recognised pathogens though non pathogenic contaminants were frequently present No attempt was made to perform capillary leucocyte counts on asphyxiated or infected infants Informed parental consent was obtained for serial study of the 35 healthy infants

For statistical analysis the mean PMNL counts of the normal group (A below) was compared with each other group using the standard error of the difference between the two means

RESULTS

(1) *Infant grouping*

The infants were divided into 4 groups based on clinical and bacteriological findings

(A) Fifty seven normal infants (160 aspi rates) showed no evidence of asphyxia or in fection All 35 studied serially were in this group

Table 1 *Comparison of all aspirates (regardless of time) mean PMNL counts in normal infected and asphyxiated infants*

Group	A Normal	B Pneumonia	C +ve Bacteriology	D Asphyxia
Number of infants	57	14	16	20
Number of aspirates	160	23	28	20
Mean PMNL % (±S D)	37 4 (±21 1)	35 3 (±26 9)	48 0 (±28 3)	29 6 (±20 2)
Significance compared with A	–	Not significant	Not significant	Not significant

(B) Fourteen infants (23 aspirates) had pneumonia diagnosed within 24 hours of birth This was a clinical and radiological diagnosis by the physicians in charge and not known to ourselves In addition to clinical and chest X ray features of pneumonia some had supportive evidence of infection viz membranes ruptured for more than 48 hours (3 cases) maternal/neonatal fever (5 cases) thick, foul smelling lochia or gastric aspirates (5 cases) Only 2 of the 14 infants however had positive aspirate cultures (*Escherichia coli* once *Streptococcus faecalis* once) None were considered to have Idiopathic Respiratory Distress Syndrome although 5 were of low birth weight (ranging from 1 7 to 2 5 kg)

(C) Sixteen infants (28 aspirates) had one or more positive cultures defined as a colony count greater than 50000 per ml of a recognised pathogen There were 19 positive cultures (7 *E coli* 3 *Streptococcus faecalis* 2 each of *Pseudomonas Enterobacter cloacae* and *Candida* 1 each *Enterococcus Proteus* and *Staphylococcus aureus*) 16 (84%) of these positive cultures were from clinically healthy infants As seen 2 infants were diagnosed as having pneumonia and were also included in Group (B) The remainder were clinically normal and none was premature

(D) Twenty infants (20 aspirates) suffered severe birth asphyxia (Apgar score less than 5 at 2 minutes) These aspirates were all collected within 1 hour of birth none had positive cultures and none were diagnosed as having pneumonia 3 were of low birth weight (ranging from 1 9–2 5 kg)

In all four groups non pathogenic contaminants (e g *Staphylococcus albus Alpha Haemolytic Streptococcus Lactobacillus*) were frequently encountered and were ignored

(2) *Percentage PMNL in different infant groups*

Table 1 shows the mean PMNL count in each group after analysing all aspirates collected from Day 0–4 regardless of time

Table 2 *Comparison of 1st aspirates only (0–1 hours after birth) mean PMNL counts in normal infected and asphyxiated infants*

Group	A Normal	B Pneumonia	C +ve Bacteriology	D Asphyxia
Number of infants	57	12	14	20
Number of aspirates	57	12	14	20
Mean PMNL % (±S D)	13 7 (±10 7)	31 0 (±26 0)	21 8 (±19 1)	29 6 (±20 2)
Significance compared with A	–	$p<0\ 05$	Not significant	$p<0\ 05$

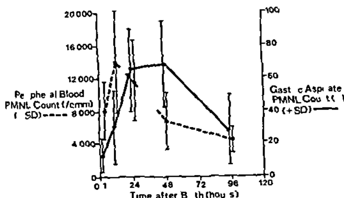


Fig 1 Influence of time on the mean PMNL counts in aspirates and blood in 35 infants studied serially after birth

There is a high mean percentage PMNL count (37 4%) in the normal group and no significant difference between groups In Table II however when only those aspirates collected at birth are analysed the PMNL counts in Group B (31 0%) and Group D (29 6%) are significantly higher than Group A (13 7%) The mean count in Group C infants (21 8%) is higher than the normal group but not significantly so

(3) Serial observations of blood and aspirate PMNL counts

In the 35 healthy infants studied serially there is a clear-cut postnatal rise and fall in both blood and aspirate counts (Fig 1) the maximum rise being between 0 and 12 hours in blood and between 12 and 24 hours in aspirates the change in aspirate counts apparently lagging 12 hours behind that seen in the blood The correlation was therefore examined between these individual infants 12 hour blood and 24 hour aspirate PMNL counts (Fig 2) The scattergram shows a significant correlation although the use of a percentage PMNL count in aspirate and absolute count in blood does not allow for strict comparison

DISCUSSION

From this study no value can be claimed for bacterial culture of gastric aspirates in screening for infection Of the 14 infants diagnosed as having pneumonia only 2 had significant cultures of pathogenic bacteria Conversely only 3 of the 19 positive aspirate cultures were from infants with pneumonia

PMNL infiltration in foetal adnexae may indicate either neonatal infection (5) or asphyxia (3) Gastric aspirate cellularity at birth may mirror these intrapartum changes but aspirates 6 hours or more after birth probably reflect the infant s lung secretions Immediate postnatal aspirate cellularity has been thought to identify infected infants but with frequent false positives after birth asphyxia (6)

We also found aspirate counts at birth significantly above normal in both pneumonia and asphyxia so that the two would not be differentiated in prolonged or premature labour where both pathologies often coexist

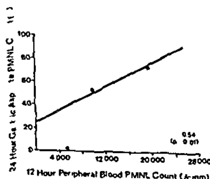


Fig 2 Scattergram showing the correlation between 12 hour blood and 24 hour aspirate PMNL count in individual infants

Later aspirates should reflect the PMNL content of the lung rather than the amniotic fluid but we have found that any pathological changes become masked by a physiological rise and fall in aspirate counts parallel to but lagging behind the blood changes Consequently when no regard is paid to time (Table I) a high PMNL percentage is found in the normal group and this is not significantly different from the pathological groups

The postnatal rise and fall in blood PMNL counts is well known (7) the effort of birth probably displaces PMNL from marginal to circulating pool as is known to occur whenever blood velocity increases (1) PMNL accumulation in aspirates has also been reported before in normal infants after birth (4) Although the nature of aspirated material makes absolute PMNL counts impossible examination of stained aspirate smears regularly gives an impression of a tremendous influx of PMNL over the first 24 hours and which gradually subsides after 48–72 hours It seems reasonable to relate this to the well established changes in absolute PMNL counts occurring in the peripheral circulation The later rise in aspirate counts we have found may be due to passage out of the peripheral PMNL pool This could be either through the bowel wall or more probably via the lung vessels since there is a constant ebb and flow of PMNL between lungs and blood and considerable lung sequestration during increased respiration (2) If neonatal aspirates reflect lung cellularity our findings reveal a physiological response to birth a time when vigorous respiration and pulmonary circulatory changes are uniquely combined The high aspirate counts found at birth in asphyxiated and infected infants suggest that their increased respiratory efforts have accelerated this process

The time interval after birth may underly the previous report of significant differences in aspirate PMNL counts between infected and non infected infants (8) The mean age of those infants was 6 days at which tim the physiological rise and fall is past an aspirate cellularity is useful in diagnosis I is less specific in the immediate postnat period ironically the time when pneumoni is most frequently suspected but rarel proved

We conclude that aspirate cellularity is useful screening test if pneumonia is sus pected at birth but must be interpreted wit caution particularly in asphyxiated infant and in any infant after the first 12 hours

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REFERENCES

1 Athens J W Raab S O Haab O P Maue A M Ashenbrucker N Cartwright G E & Wi trobe M M Leukokinetic studies III The distr bution of granulocytes in the blood of normal sul jects *J Clin Invest 40* 989 1961
2 Bierman H R Kelly K H Cordes F L Petr kis N L Kass H & Shpil E L The influenc of respiratory movements upon the circulating leuc cytes *Blood 7* 533 1952
3 Maudsley R F Brix G A Hinton N A Ro bertson E M Bryans A M & Haust M D Placental inflammation and infection *Am J Obste Gynecol 95* 640 1966
4 Mims L C Medawar M S Perkins J R & Grubb W R Predicting neonatal infections by eval uation of the gastric aspirates A study of 207 pa tients *Am J Obstet Gynecol 114* 232 1972
5 Overbach A M Daniel S J & Cassaday G The value of umbilical cord histology in the manage ment of potential perinatal infection *J Pediatr 76* 22 1970
6 Ramos A & Stern L Relationship of prematur rupture of the membranes to gastric fluid aspirati in the newborn *Am J Obstet Gynecol 105* 1247 1969
7 Xanthou M *Leucocyte blood picture in* healthy full term and premature babies during the neonatal period *Arch Dis Child 45* 242 1970
8 Yeung C Y & Tam A S Y Gastric aspirat findings in neonatal pneumonia *Arch Dis Child 47* 735 1972

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BLOOD GLUCOSE AND PLASMA INSULIN AND GLUCAGON RESPONSE DURING INTRAVENOUS GLUCOSE TOLERANCE TEST IN NEWBORN INFANTS AFFECTED BY ERYTHROBLASTOSIS FOETALIS

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From the Paediatric Clinic of the University of Perugia[1] and the Service of Neonatal Pathology of the University of Milan[2] Italy

ABSTRACT Massi Benedetti F Marini A Caccamo M L. and Falorni A (Paediatric Clinic of the University of Perugia and the Service of Neonatal Pathology of the University of Milan Italy) Blood glucose and plasma insulin and glucagon response during intravenous glucose tolerance test in newborn infants affected by erythroblastosis foetalis Acta Paediatr Scand 64 113 1975 —Intravenous glucose injection (1 g/kg b w) was performed in eight newborn infants affected by erythroblastosis foetalis (IEF) and in seven controls during the first day of life in order to study insulin and glucagon response The IEF infants were affected by mild or moderate hemolytic disease and their blood glucose values and plasma insulin concentrations before and throughout the test did not differ significantly from those of the controls. After the glucose injection the plasma glucagon concentrations showed great variations in both groups The control infants did not show any significant changes in the IEF infants significant decreases were seen at 3 and 20 min of the test These data seem to indicate that the alpha-cell sensitivity to glucose is greater in IEF than in normal infants and is not dependent on the development of the glucose-mediated insulin release mechanism

KEY WORDS Newborn infant erythroblastosis foetalis glucose metabolism insulin glucagon

Hypoglycaemia which occurs in erythroblastosis foetalis has been attributed to beta-cell hyperactivity Evidence of this has been furnished by the increase of the insulin content in the pancreas (3) and the insulin concentration in the plasma (1 7 19) and the urine (18) Recently a more active glucose mediated insulin release has been described in newborn infants affected by hemolytic disease (5 18) Milner et al (16) have reported higher plasma glucagon concentrations in erythroblastotic infants when compared with normal newborn infants As insensitivity of the alpha-cell to glucose has been recently reported in human infants (14 15 17) as well as in the rat foetus and in its neonate (4) plasma glucagon and insulin response to glucose injected intravenously in erythroblastotic infants was studied and compared with that of non erythroblastotic newborns

MATERIAL AND METHODS

Fifteen full term newborn infants were selected Eight infants were affected by erythroblastosis foetalis due to Rh immunization (IEF) and their clinical features are reported in Table 1 all of them received exchange transfusions for the hemolytic disease The erythroblastosis was found to be mild or moderate according to Smith s criteria (21) Seven newborn infants 2 of them with mild and transient respiratory difficulty at birth 2 presenting a fractured clavicle 2 presenting a cleft palate and 1 with imperforate anal membrane accepted in the Neonatal care Unit of the Paediatric

Table 1 *Clinical features of infants with erythroblastosis foetalis*

Infant no	Sex	Birth weight (g)	Gestational age (weeks)	Delivery: Method	Delivery: Glucose to mother	Age and measurements: Age (hr)	Hb (g/100 ml)	Ht	Serum bilirubin (mg/100 ml)
1	F	3 250	38	Vaginal	+	16	23 5	63%	9 5
2	M	2 700	37	Vaginal	–	12	14 9	45%	17 0
3	M	2 830	37	Vaginal	–	18	13 1	42%	14 0
4	F	2 550	37	Cesarean	–	14	12 5	40%	15 5
5	F	3 250	39	Vaginal	+	16	14 2	43%	14 5
6	F	2 600	38	Vaginal	–	14	15 0	44%	10 0
7	F	2 850	38	Vaginal	+	18	13 7	42%	14 8
8	F	3 150	38	Vaginal	+	15	15 2	44%	11 2

Clinic of Perugia were selected as controls no severe disease being evidenced during the hospitalization their birth weights ranged from 2 750 g to 3 850 g and their gestational ages ranged from 38 to 40 weeks Family histories were negative for diabetes mellitus in each case studied Four mothers of the control infants were given glucose during labor All the control infants were born by vaginal delivery

In all cases glucose was injected intravenously (1 g/kg/body weight during 30 seconds) into the umbilical vein using a catheter and by a technique described elsewhere (5) Hypoglycemic levels were never found during serial glucose estimation in the blood of any of the cases before the tolerance tests were made The test was performed between 6 and 18 hours of life before any feeding started and in the erythroblastotic infants also before exchange transfusions The catheter was used for blood glucose administrations and blood sampling after rinsing with saline Samples of blood were taken for determination of glucose insulin and glucagon at 0 3 5 10 20 30 40 60 and 90 minutes 0 1 ml of blood was immediately deproteinized in uranyl acetate for glucose determination the remainder was centrifugated at +4 C and the plasma was stored at −20°C until the insulin and glucagon assay was carried out

Blood glucose was estimated by a glucose oxidase method (11) using reagents from Boehringer Mannheim G m b H glucose tolerance was determined by plotting glycemic values on semilogarithmic paper and calculating the disappearance rate (K_t) according to the method of Conard et al (2) Plasma insulin was measured by the immunoassay method of Hales & Randle (10) using CEA IRE Sorin (Saluggia Vercelli Italia) commercial kits The plasma glucagon was measured by immunoassay (12) using a porcine glucagon I[131] (donated by C N T S Paris) and *antiglucagon anti serum 30 K* (furnished by Dr Unger Dallas) which is highly specific for pancreatic glucagon

All statistical calculations were performed by the Student *t* test and linear regression analysis

RESULTS

Control infants

The mean values (±1 S D) of fasting blood glucose before the i v GTT were performed and of K_t are reported in Table 2 The mean values of plasma insulin levels (±1 S E) before and after intravenous glucose injection are reported in Fig 1 The double peak pattern of insulin response to glucose previously described (5 6) was present in

Table 2 *K_t and fasting glycemic values before the i v glucose injection in erythroblastotic and control infants*

No of subjects	Fasting blood glucose (mg/100 ml): Mean	±1 S D	Range	Glucose tolerance expressed as K_t: Mean	±1 S D	Range
Non erythroblastotic infants						
7	46 2	18 5	35–70	1 12	0 44	0 38–1 82
Erythroblastotic infants						
8	44 3	16 5	36–67	1 29	0 54	0 76–2 10

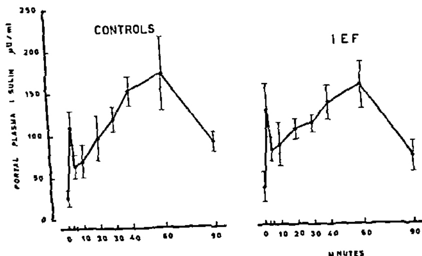


Fig 1 Plasma insulin levels (mean ±1 S E) during intravenous glucose tolerance test (1 g/kg/body weight) in erythroblastotic and control infants

all the cases studied The data on plasma glucagon levels before and after the intravenous glucose injection are reported in Table 3 In the non-erythroblastotic infants the plasma glucagon concentrations changed only slightly and insignificantly during the whole i v GTT

Infants with erythroblastosis foetalis

The mean values of fasting blood glucose and K_t (Table 2) were not significantly different from those of the control group Also the mean plasma insulin levels at fasting and at any time of the i v GTT were not significantly different from those of the non-erythroblastotic infants (Fig 1) All the IEF infants showed the biphasic pattern of plasma insulin response to glucose injected intravenously The mean basal plasma glucagon concentration in the IEF was very similar to that of the control infants (Table 3) but after the glucose injection an evident decrease was observed In comparison with the control group the IEF showed mean plasma glucagon levels significantly lower at 3 ($p<0.05$) and 20 ($p<0.01$) minutes of the i v GTT

The following correlations were studied

(i) hematocrit and serum bilirubin *vs* fasting blood glucose K_t plasma insulin and glucagon concentrations before and throughout the i v GTT

(ii) fasting blood glucose *vs* fasting plasma insulin and glucagon

(iii) K_t *vs* plasma insulin and glucagon levels before and throughout the i v GTT

(iv) plasma insulin *vs* plasma glucagon levels before and throughout the i v GTT

(v) birth weight *vs* K_t and plasma insulin levels at fasting and throughout the i v GTT

No significant correlation was found in any of these statistical analyses in the two groups of infants

DISCUSSION

Only infants affected by mild or moderate erythroblastosis foetalis were selected for the present research In such newborns fasting blood glucose K_t value and plasma insulin levels before and during the i v GTT were not significantly different from those of the non-erythroblastotic infants In a previous report concerning the glucose mediated insulin release in erythroblastosis foetalis (5) a beta-cell hyperactivity was evident only in the most severe cases while the mildly affected infants showed a glucose stimulated insulin secretion pattern not un

Table 3 *Plasma glucagon levels (pg/ml) after intravenous glucose injection (1g/kg of body weight) in nonerythroblastotic infants (upper panel) and erythroblastotic infants (lower panel)*

Case no	Time (min) 0	3	5	10	20	30	40	60	90
Non erythroblastotic infants									
1	561	618	478	373	428	694	935	373	533
2	325	412	298	297	458	389	490	435	343
3	252	160	228	370	252	298	206	275	252
4	115	92	160	228	160	46	92	160	183
5	370	447	480	320	453	400	213	400	–
6	250	412	–	228	336	398	–	202	–
7	432	404	528	366	–	350	–	530	–
Mean	322	363	354	305	348	366	385	339	378
±1 S D	143	179	147	59	122	190	338	132	157
Erythroblastotic infants									
1	320	187	–	214	160	240	267	187	160
2	133	53	213	187	133	–	267	213	373
3	366	252	229	91	183	206	137	69	130
4	373	293	160	373	213	240	90	400	240
5	264	198	132	198	–	–	–	132	198
6	462	330	330	330	264	297	330	396	330
7	297	183	–	–	252	–	275	194	183
8	192	115	–	–	115	–	115	192	77
Mean	301	201	213	232	189	198	210	213	211
±1 S D	105	91	76	103	57	113	97	123	100
t	0 39	2 26	1 92	1 59	3 09	1 23	1 31	1 91	1 61
p	N S	<0 05	N S	N S	<0 01	N S	N S	N S	N S

like that of the controls However such data are not in agreement with a recent study (18) which reported the opposite the most severely Rhesus immunized infants showed plasma insulin levels after glucose injection lower than those of the mildly immunized ones moreover significant positive correlations were found in the erythroblastotic infants between birth weight and fasting insulin concentration and between birth weight and K_t value

At present it is impossible to explain the apparent disagreement among the various results and further research will be necessary to elucidate this point However, the following differences in the research conditions used by Mølsted Pedersen et al (18) and those of the present research should be noted (*a*) Mølsted Pedersen et al (18) began their study of the insulin response to glucose only at the seventh minute of the test Falorni et al (5) have shown that it is principally during the first 5 minutes of the test that the insulin response to glucose varies according to the gravity of the hemolytic disease (*b*) moreover Mølsted Pedersen et al gave greater uniformity to their investigation by performing the test in each case at the third hour of life in the present work the hour of the test varied during the first day of life This latter aspect may explain why no significant correlation was found between the birth weight the K_t value and the fasting plasma insulin level in the present investigation

With regard to the mean plasma glucagon level at fasting of the IEF this did not differ from that of the control infants This finding cannot be compared to the data reported ˙ Milner et al (16), for their in

fants aged from 8 to 93 hours of life and some of which had already started feeding

After glucose injection the plasma glu cagon concentrations of the control infants did not show significant changes The alpha cell insensitivity to glucose of the neonatal pancreas has been previously reported in the human (14 15) as well as in the animal (4 8) however plasma glucagon was found to be reduced by glucose administration in in fants born to diabetic mothers and showing high plasma insulin levels (14 15) as well as in normal infants receiving glucose plus exogenous insulin (15) In view of data suggesting that the alpha cell response to glu cose is an insulin-dependent process (22) such insensitivity has been suggested to be a consequence of the low glucose mediated insulin release in the neonate (6 9)

In IEF a significant decrease of plasma glucagon was observed being evident 3 minutes after the glucose injection and showing a polyphasic pattern during the i v GTT However such a decrease did not appear to be correlated with beta-cell hyper activity the erythroblastotic infants of this research not showing plasma insulin levels during the i v GTT different from those of the non erythroblastotic infants Although t present there is no way of interpreting his finding it does seem to imply the fol owing

(1) The inhibition of alpha-cell activity by glucose is probably not an entirely insulin dependent mechanism as suggested also by studies performed in diabetic subjects (13)

(2) It is not yet known how the hemolytic disease influences the endocrine pancreas development Histological findings have demonstrated that the islet hyperplasia is dependent upon the beta cell as well as the alpha-cell proliferation (23) It might be sug gested therefore that the alpha-cell sensitivity to glucose in IEF develops more rapidly and without a close dependence on the develop ment of the glucose mediated insulin release mechanism

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REFERENCES

1 Barret C T & Oliver T K Jr Hypoglycemia and hyperinsulinism in infants with erythroblastosis fetalis *N Engl J Med 278* 1260 1968

2 Conard V Franckson J R M Bastenie P A Kestens J & Kovacs L Etude critique du triangle d hyperglycaemie intraveineux chez l homme normal et détermination d un coeffi cient d assimilation glucidique *Arch Int Phar macodyn 93* 277 1951

3 Driscoll S G & Steinke J Pancreatic insulin content in severe erythroblastosis *fetalis Pediat rics 39* 448 1967

4 Edward J C Asplund K & *Lundqvist G Glu* cagon release from the pancreas of the newborn rat *J Endocr 54* 493 1972

5 Falorni A Fracassini F Massi Benedetti F & Amici A Glucose metabolism plasma *insulin* and growth hormone secretion in newborn infants with erythroblastosis fetalis compared with normal new borns and those born to diabetic mothers *Pediat rics 49* 687 1972

6 Falorni A Fracassini F Massi Benedetti F & Maffei S Glucose metabolism and insulin secre tion *in the* newborn infant—Comparison between the responses observed at the first and the seventh day of life to intravenous and oral glucose tolerance tests *Diabetes 23* 17? 1974

7 From G L Driscoll *S G* & Steinke J Serum insulin in *newborn* infants with *erythroblastosis* fetalis *Pediatrics 44* 549 1969

8 Girard J Assan R & Jost A Glucagon *in* the rat foetus *In* K S Comline G S Dawes and P W Nathanielsz *(eds) Foetal and neonatal physiology* Cambridge Univ Press 1973 *p 456*

9 Grasso S Messina A Saporito M & Reitano *G* Serum insulin *response* to glucose and amino-acids *in the* premature infant *Lancet II* 755 1968

10 Hales C N & Randle P J Immunoassay of in sulin with insulin antibody precipitate *Biochem J 88* 137 1963

11 Huggett A S & Nixon D A Use of glucose oxidase peroxidase and O-dianisidine in the deter mination of blood and urinary glucose *Lancet II* 368 1957

12 Leclerq Meyer V Mihale P & Malaisse W J Une méthode de dosage radioimmunologique du glucagon comportant une séparation par le charbon dextran *Diabetologia 6* 121–129 1970

13 Lefèbvre P J & Unger R H *Glucagon molecu lar physiology clinical and therapeutic implications* Pergamon Press Oxford 1972 p 250

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HIGH VOLTAGE QRS COMPLEXES IN CHILDREN WITH INNOCENT VIBRATORY HEART MURMUR

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ABSTRACT Michaelsson M and Tuvemo T (Department of Paediatrics University Hospital Uppsala Sweden) High voltage QRS complexes in children with innocent 'vibratory heart murmur Acta Paediatr Scand 64 119 1975.—The electrocardiographic findings in 86 four year-old children with innocent murmur of the so-called vibratory type are described The electrical axis the Q R and S waves in the right and left precordial leads were compared with figures from the literature and with a control group of 4-year-old children without signs of somatic disease The most significant findings were higher amplitudes of Q and R waves over the left precordium in children with vibratory murmur Many children in this group had amplitudes of these waves exceeding accepted normal limits There were no findings compatible with organic heart disease and the difference as compared to normal values and controls is probably a matter of variations in the position of the heart

KEY WORDS Innocent heart murmur functional heart murmur vibratory heart murmur ECG ECG criteria of ventricular hypertrophy QRS amplitudes four year-old children

Increased amplitude of the QRS complexes is used as an important criterion in the electrocardiographic diagnosis of ventricular hypertrophy We have often observed increased left precordial voltages in children having an innocent murmur of the so-called vibratory type [1]

To our knowledge this finding has not been described in the literature The ECG of children with vibratory murmurs has therefore been more systematically studied

In the paediatric age groups the magnitude of the voltages is highly variable within and between the various age groups Moreover the range of QRS precordial amplitudes is highly variable from series to series in the normal materials published As an example the maximal amplitude of R in lead V_6 in children of about 4 years is 28 19 26 23 and 21 mm (1 2 3 4 5) in five various publications It cannot be excluded that the differences between normal materials is explained by variations in the technique of registration and therefore the ECG recordings from children with vibratory murmurs were compared not only with figures from the literature but also with an own normal material

[1] Vibratory murmur is generally defined as a very distinctive vibratory or twanging string or buzzing murmur with a uniform medium frequency as seen on the phonocardiogram It begins shortly after the first sound and its duration and intensity may vary from beat to beat usually being early or midsystolic It may be heard over the whole precordium but is usually best audible along the left sternal edge in the third to fourth interspace

MATERIAL

The material of children with innocent murmurs consists of 86 cases between 4 and 5 years of age 52 were boys and 34 were girls They were referred to

14 Luycks A S Massi Benedetti F Falorni A & Lefébvre P J Presence of pancreatic glucagon in the portal plasma of human neonates Differences in the insulin and glucagon responses to glucose between normal infants and infants from diabetic mothers *Diabetologia 8* 296 1972
15 Massi Benedetti F Luycks A Fracassini F Lefébvre P J & Falorni A Glucose metabolism and insulin glucagon relationship in newborn infants of normal and diabetic mothers Abstracts of the *Meeting of the European Association for the Study of Diabetes* Madrid 1972
16 Milner R D G Fekete M & Assan R Glucagon insulin and growth hormone response to exchange transfusion in premature and term infants *Arch Dis Childh 47* 186 1972
17 Milner R D G Fekete M Assan R & Hodge J S Effect of glucose on plasma glucagon growth hormone and insulin in exchange transfusion *Arch Dis Childh 47* 179 1972
18 Molsted Pedersen L Trautner H & Jorgensen K R Plasma insulin and K values during intravenous glucose tolerance test in newborn infants with erythroblastosis foetalis *Acta Paediatr Scand 6?* 11 1973
19 Raivio K O & Österlund K Hypoglycemia and hyperinsulinemia associated with erythroblastosis fetalis *Pediatrics 43* 217 1969
20 Schiff D & Lowy C Hypoglycemia and excretion of insulin in urine in hemolytic disease of the newborn *Pediatr Res 4* 280 1970
21 Smith C H *Blood disease of infancy and childhood* St Louis Mo Mosby 1966 p 133
22 Unger R H Aguilar Parade E Muller W & Eisentraut A M Studies of pancreatic alpha-cell function in normal and diabetic subjects *J Clin Invest 49* 837 1970
23 Van Assche F A De Gasparo M Renaer M & Gepts W The endocrine pancreas in erythroblastosis fetalis *Biol Neonat 15* 176 1970

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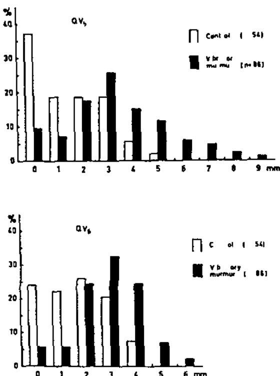


Fig 1 The amplitudes of Q waves in leads V_5 and V_6 in the vibratory murmur group as compared with the control group

DISCUSSION

The electrocardiogram of children with vibratory murmurs is different as compared to the control material The amplitudes of Q in leads V_5 and V_6 and of R in leads V_5 and V_6 are higher Not rarely the voltage is so high that a left ventricular hypertrophy is suspected On the other hand the amplitude of S in the right precordial leads is somewhat lower as compared to the control group resulting in a lack of difference of the sum of R in left precordial and S in right precordial leads The position of the transitional zone is the same in the two materials Clockwise rotation of the heart could thus not explain the difference in the left precordial amplitudes The picture of all the precordial leads in children with vibratory murmurs is not typical for a left ventricular hypertrophy It is more probable that the findings are explained by a shift in position decreasing the distance between the apex of the heart and the precordium The finding of deep Q waves and high R waves in the left precordial leads in otherwise healthy children with vibratory murmurs support the views found in the literature (6 7) that

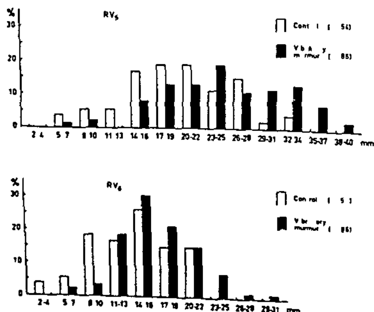


Fig 2 The amplitudes of R waves in leads V_5 and V_6 in the vibratory murmur group as compared with the control group

Table 1 *Electrocardiographic findings in children with vibratory heart murmur as compared with control*

	Mean controls	Mean vibr	σ controls	σ vibr	S E M controls	S E M vibr	t obs	
El axis	56 02	64 47	23 30	21 78	3 17	2 35	2 17	$p<0\ 05$
QV_5	1 43	3 34	1 40	2 03	0 19	0 22	6 08	$p\leq0\ 001$
QV_6	1 65	2 90	1 26	1 30	0 17	0 14	5 61	$p\leq0\ 001$
RV_1	8 91	7 72	3 00	3 44	0 41	0 37	−2 08	$p<0\ 05$
RV_5	19 61	24 80	6 31	7 12	0 86	0 77	4 38	$p\leq0\ 001$
RV_6	13 76	16 42	4 62	4 49	0 63	0 48	3 37	$p<0\ 001$
SV_1	11 35	9 91	5 02	5 06	0 68	0 55	−1 65	n s
SV_5	4 91	4 28	2 94	3 34	0 40	0 36	−1 13	n s
SV_6	1 56	1 43	1 40	1 46	0 19	0 16	−0 50	n s
RV_6+SV_1	25 11	26 33	6 53	6 98	0 89	0 75	1 03	n s

the department of paediatrics for evaluation of the systolic murmur noted at routine health examination The history did not reveal any illness of significance and the findings at the physical examination were normal The murmur was a typical vibratory systolic murmur at auscultation which was also confirmed at phonocardiography The blood pressure was normal No ECG had been recorded before the admission

The control material consists of 54 children between 4 and 5 years of age 26 were boys and 28 were girls These children had been referred to the department of paediatrics from routine health examinations because of socio-medical or psychological problems The history in the control group did not reveal any somatic illness of significance A comparison of the weights and lengths between the groups did not show any significant difference

METHODS

A standard 12 lead electrocardiogram was recorded on a direct writing apparatus (Mingograf 81 Elema) at 50 mm/sec paper speed with a calibration of 1 mV =10 mm Phonocardiograms were recorded with the same apparatus using the standard phonopreamplifier The recordings were made over the second right intercostal space over the second third and fourth left intercostal space parasternally and over the apex For the recordings frequency filters with nominal frequences of 12 5 25 100 400 cycles/sec and one with auditory amplification were used The paper speed was 100 mm/sec

The amplitudes of Q R and S in lead V_1 V_5 and V_6 were registered The voltage was calculated from the upper margin of the isoelectric line to the tip of the R wave and from the lower margin to the tip of the S wave respectively The electrical axis was calculated from standard leads I and III The recordings were also studied with respect to the occurrence of any other abnormalities regarding rate and rhythm P wave and PR interval ventricular activation time intraventricular conduction transitional zone and ST segment and T waves No such abnormalities were found in the test group or in the control group

RESULTS

The results from the comparison with the own normal material are summarized in Table 1 Especially the amplitude of Q in leads V_5 and V_6 and R in leads V_5 and V_6 are significantly higher in the vibratory murmur group The mean values of S and R in lead V_1 are smaller in the vibratory murmur group but the difference is of questionable significance The electrical axis has a somewhat higher numerical value in the vibratory murmur group The distribution of the amplitudes of the Q waves in leads V_5 and V_6 is shown in Fig 1 In the control group 90% of the cases have amplitudes of 3 mm or less while in the vibratory murmur group 40% and 30% respectively have amplitudes of 4 mm or higher in leads V_5 and V_6 In the material of Alimurung et al (1) consisting of 124 children between 3 and 5 years of age 90% have an amplitude of Q in V_5 of 3 5 mm or less and amplitude of Q in V_6 of 2 5 mm or less According to the same authors the maximal amplitude of R in V_5 and V_6 is 25 and 19 mm or less in 90% of the cases In the vibratory group here described 44% and 25% respectively had amplitudes of more than 25 and 19 mm (Fig 2) The differences shown in the comparison with the own normal material can thus be further verified by the comparison with earlier published normal materials

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GLYCOSIDASES IN HUMAN SKIN FIBROBLAST CULTURES

α-fucosidase α-galactosidase α-glucosidase β-mannosidase and N acetyl α-glucosaminidase

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From the Department of Clinical Chemistry University Hospital Lund Sweden

ABSTRACT Hultberg B Sjoblad S and Öckerman P A (Department of Clinical Chemistry University Hospital Lund Sweden) Glycosidases in human skin fibroblast cultures Acta Paediatr Scand 64 123 1975 —Five glycosidases α fucosidase α galactosidase α-glucosidase β mannosidase and *N* acetyl-α glucosaminidase were studied in human skin fibroblast cultures The pH dependency kinetic properties of the enzymes and results of isoelectric focusing and ion exchange chromatography are presented Techniques suitable for diagnosing inborn lysosomal diseases in skin fibroblast cultures are defined

KEY WORDS Fibroblast cultures glycosidases Inborn lysosomal diseases

Enzyme assays on cultured human fibroblastic cell lines have recently attracted much interest in the study of inborn errors of metabolism When designing a suitable diagnostic method knowledge of the kinetic properties of the enzymes and of the existence of isoenzymes or different molecular forms often is of great and sometimes decisive importance

In an earlier investigation (13) we studied the properties and behaviour of β galactosidase (β D galactoside galactohydrolase EC 3 2 1 23) β glucosidase (β D glucoside glucohydrolase EC 3 2 1 21) α mannosidase (α D mannoside mannohydrolase EC 3 2 1 24) N acetyl β glucosaminidase (2 acetamido 2 deoxy β D glucoside acetamidodeoxy glucohydrolase EC 3 2 1 30) and β glucuronidase (β D glucuronide glucuronohidrolase EC 3 2 1 31) in human skin fibroblast cultures

These five enzymes are all connected to serious inborn lysosomal storage disorders In the present investigation we have proceeded with five more acid hydrolases known to have or possibly have relationship to inborn lysosomal disorders These enzymes are *N* acetyl α glucosaminidase (2 acetamido 2-deoxy α D glucoside acetamido deoxy glucohydrolase EC 3 2 1 50) known to be deficient in Sanfilippo B disease (9 20) α fucosidase (α L fucoside fucohydrolase EC 3 2 1 51) deficient in fucosidosis (8) α galactosidase (α D galactoside galactohydrolase EC 3 2 1 22) deficient in Fabry s disease (5 14) α glucosidase (α D glucoside glucohydrolase EC 3 2 1 20) deficient in Pompe s disease (11) and finally β mannosidase (β D mannoside mannohydrolase EC 3 2 1 25) The latter enzyme has only recently been described in man (1 4) and no enzyme deficiency is at least not yet known

The main object of the present study has been to characterize the properties of the enzymes in human skin fibroblastic cell lines and to design methods suitable for diagnosing inborn lysosomal disorders taking into account kinetic properties and the possible existence of isoenzymes or different molec

voltage criteria should be utilized with considerable caution in diagnosing left ventricular hypertrophy When precordial voltage criteria alone are used as an index of left ventricular hypertrophy it seems wise to follow the advice given by Walker et al (7) They state that a reliable index of left ventricular hypertrophy is present when the SV + RV_5 value is greater than 60 mm in children less than 11 years of age or greater than 55 mm in females and 65 mm in males past 11 years of age These figures are higher than those given in many publications Considering the inaccuracy of precordial voltage criteria alone as an index of ventricular hypertrophy the addition of vector loop analysis might possibly be of practical importance

REFERENCES

1 Alimurung M M Joseph L G Nadas A S & Massell B F Unipolar precordial and extremity electrocardiogram in normal infants and children *Circulation 4* 420 1951
2 Keck E W *Pädiatrische Kardiologie* Urban & Schwarzenberg München 1972 p 14
3 Heck W & Stoermer J *Pädiatrischer EKG Atlas* Thieme Stuttgart 1959 p 19
4 Wasserburger R H *The normal and abnormal unipolar electrocardiogram in infants and children* Williams & Wilkins Baltimore 1963 p 19
5 Ziegler R F *Electrocardiographic studies in normal infants and children* Thomas Springfield Ill 1951 p 157
6 Traywick J P Maron B J Schubert K & Krovetz L J The electrocardiographic diagnosis of left ventricular hypertrophy in apparently normal children *J Pediatr 83* 201 1973
7 Walker C H M & Rose R L Importance of age sex and body habitus in the diagnosis of left ventricular hypertrophy from the precordial electrocardiogram in childhood and adolescence *Pediatrics 28* 705 1961

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The main object of the present study has been to characterize the properties of the enzymes in human skin fibroblastic cell lines and to design methods suitable for diagnosing inborn lysosomal disorders taking into account kinetic properties and the possible existence of isoenzymes or different molec

ular forms Only a thorough knowledge of the enzymes in fibroblast cultures will avoid making such diagnostic mistakes in future cases as may otherwise be made when per forming enzyme assays on fibroblasts by directly applying methods worked out for other tissues

MATERIAL AND METHODS

Controls

As controls were used young adults of both sexes and also children hospitalized for various illnesses not apparently influencing their general health Minor age and sex differences cannot be excluded but would not influence the conclusions drawn in this study

Patients

Case 1 Pompe s disease Male 10 years old Slowly progressive muscular weakness first noted at the age of 2 and advanced to the stage that the patient needs assistance by a respirator Muscle biopsy has shown marked vacuolisation with storage of PAS positive ma terial Chemical assay gave the following results gly cogen 14% of wet weight (upper normal limit in this laboratory 2%) α glucosidase less than 0 005 IU/g wet weight (controls 0 03–0 15) phosphorylase 21 IU/g wet weight (controls 60–140) amylo-1 6 glucosidase 0 15 IU/g wet weight (controls 0 20–0 70)

Case 2 Cystic fibrosis Male 13 years old He had symptoms of chronic pulmonary disease and of mild intestinal insufficiency The biochemical findings were increased electrolyte concentrations in sweat (pilocarpin test) and decrease of pancreatic enzymes

Case 3 Sanfilippo s disease Male 15 years old The obvious clinical signs of mucopolysaccharidosis were extremely vague except for progressive mental retarda tion The urinary excretion of glycosaminoglycans was abnormal 29 mg glucuronic acid/24 hours (age matched controls <12) Analysis by cellulose acetate elec trophoresis and of eluate from an ecteola column showed dominance of heparan sulphate (25)

Case 4 Sanfilippo s disease Female 9 years old Sister of case 3 No obvious abnormal clinical signs except grave mental retardation with an IQ below 40 Glycosaminoglycans in urine correspond to 14 mg glu curonic acid/24 hours (age matched controls < 9) As in her brother heparan sulphate was the dominating glycosaminoglycan excreted

Cell culture

Biopsies were taken from the extensor surface of the upper arm and established in cultures similar to those of earlier workers (10) Cells were maintained in 30 ml culture bottles (Falcon Plastics Los Angeles) The medium used was Eagle MEM (Flow Labs Irvine Scotland) with Hanks salts 20% newborn calf serum penicillin 100 IE/ml and streptomycin 100 IE/ml

At the stage of confluent monolayer cells were re moved by trypsinisation (0 25% trypsin solution Flow Labs) centrifuged at 1500 g/min for 5 min at room temperature washed twice with saline and then frozen at −20°C until analysis (not more than 2 weeks later) No important change of enzyme activity occurred during this time

Enzyme assays

Enzyme assays were performed on crude homogenates if not otherwise stated Common to all methods was that the pH of the incubation mixture was the pH optimum for the enzyme and substrate used The con centration of the substrate was not always optimal the solubility of some substrates not being large enough In all methods the enzyme activity measured was directly proportional to the amount of tissue in the incubation tube Non-enzymatic hydrolysis of the substrates used was negligible Protein was assayed according to Lowry et al (18)

α-fucosidase was assayed with *p* nitrophenyl-α-L fucopyranoside (Koch Light Laboratories Colnbrook England) 8 mM 150 μl and citrate buffer 1 M pH 5 5 150 μl using 50 μl of fibroblast homogenate Incubation times were 0 1 8 and 24 hours at 37°C The reaction was stopped by adding 150 μl of 5% TCA The tube was centrifuged and 0 2 ml of super natant was mixed with 3 ml of glycine buffer 0 2 M pH 10 7 (containing glycine 0 2 M Na_2CO_3 0 175 M and NaCl 0 1 M) Liberated *p* nitrophenol was meas ured at 400 nm with *p* nitrophenol (Sigma Chemical Company St Louis) as standard

α galactosidase was assayed with 4 methylumbelli feryl-α D galactopyranoside (Koch Light) 7 5 mM 100 μl and acetate buffer 1 M pH 4 5 25 μl using 25 μl of fibroblast homogenate Tubes were incubated for 0 30 and 60 min at 37 C The enzyme activity was stopped by the addition of 3 ml of glycine buffer and fluorescence was read in an Aminco Bowman spectrofluorimeter with exciting wavelength 365 nm and emitting wave length 448 nm 4-methylumbelliferone (Koch Light) in glycine buffer was used as standard

α glucosidase was assayed with 4 methylumbelli feryl-α D glucopyranoside (Koch Light) 1 mM 100 μl and citrate buffer 0 2 M 25 μl using 25 μl of fibroblast homogenate Two different citrate buffers were used one at pH 4 3 for the acid α glucosidase and one at pH 5 8 for the neutral enzyme Tubes were incubated for 0 30 60 and 120 min at 37°C The reaction was terminated and read as for α galactosidase

β mannosidase was assayed with p nitrophenyl-β D mannopyranoside (Koch Light) 16 mM 150 μl and citrate buffer 1 M pH 4 5 150 μl using 50 μl of fibro- blast homgenate Tubes were incubated for 0 1 6 and 24 hours at 37°C After incubation the same procedure was followed as for α fucosidase

N acetyl-α glucosaminidase was assayed with phenyl α N acetyl D-glucosamine (Sigma) 25 mM 100 μl and citrate buffer 1 M pH 4 5 50 μl using 100 μl of fibro

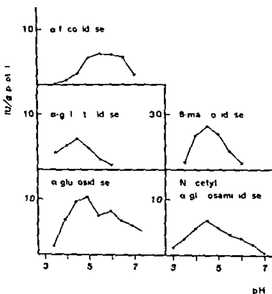


Fig 1 pH-depencency of glycosidases in crude human fibroblast homogenates Buffers used are described in Methods 1 U expressed as nmoles/min

blast homogenate Tubes were incubated for 0 1 6 and 24 hours at 37°C After incubation 250 μl of Fohlin Ciocalteau reagent (KEBO Stockholm) was added After centrifugation 700 μl of supernatant was mixed with 2 ml of Na_2CO_3 0 4 M and optical density was read after 30 min at 650 nm

N-acetyl-β glucosaminidase was assayed as earlier described (13)

RESULTS

Very varying results were seen when the pH-dependency of the enzyme activities in crude homogenates was tested (Fig 1) α fucosidase had a broad optimum at pH 5 0–6 5 α galactosidase had a more distinct maximum at pH 4 5 The same results were seen when α fucosidase was measured with 0 1 M citrate phosphate buffer or α galactosidase in 1 M citrate buffer β mannosidase showed pH-optimum at pH 4 5 In contrast α glucosidase had a biphasic curve with maxima at pH 4 3–4 8 and 5 8 The relation between the activity of α glucosidase at pH 4 3–4 8 and that at pH 5 8 was varying In some instances the more neutral activity was dominating over the acid activity and in others *vice versa* Also *N* acetyl α glucosaminidase had a broad possibly biphasic pH-curve with optimum at pH 4 5 and possibly another at pH 5 5–6 0

In Table 1 are shown the apparent Michaelis Menten constants obtained on the crude homogenates and the per cent of theoretical V_{max} obtained at the actual substrate concentration α fucosidase showed substrate inhibition when higher concentration than in our method was used

Enzyme activity measured was proportional to time within the limits given in Methods and to amount of protein in each test tube

Total activity

The activity of five glycosidases when assayed in crude fibroblast homogenates as described in Methods is shown in Table 2 In the controls mean value and observed range is given A relatively broad range was observed and it is not possible to say whether there is a Gaussian or skewed distribution For that reason no standard deviation is given

Table 1 *Apparent Michaelis Menten constants and per cent of theoretical maximum activity (V_{max}) obtained at the actual substrate concentration* [s]

Enzyme	pH	Apparent K_m	Per cent of V_{max}	[s]
α fucosidase	pH 5 5	0 42 mM	80	3 4 mM
α-galactosidase	pH 4 5	5 0 mM	50	5 0 mM
α-glucosidase	pH 4.3	0 90 mM	45	
	pH 5 8	0 33 mM	65	0 67 mM
β-mannosidase	pH 4 5	1 7 mM	85	6 86 mM
N-acetyl-α-glucosaminidase	pH 4 5	0 44 mM	82	10 0 mM

Table 2 *Activity of glycosidases in cultured fibroblasts*

Activity expressed as IU/g protein in the controls as mean value (observed range)

Diagnosis	Sex and age	Sub culture	α fuco sidase	α galacto sidase	α glucosidase pH 4 3	α glucosidase pH 5 8	β manno-sidase	N acetyl α glucos aminidase
Controls (*n* = 10)	Young adults Both sexes	P1–P3	0 80 (0 33–2 03)	0 45 (0 22–0 65)	0 75 (0 17–2 78)	0 60 (0 23–1 60)	0 72 (1 80–6 80)	0 81 (0 23–1 36)
1 Pompe s disease	M 10	P1	0 78	–	0 06	0 30	3 29	1 13
		P3	0 71	–	0 02	0 38	6 76	1 33
2 Cystic fibrosis	M 13	P1	0 54	–	0 50	0 56	1 85	0 78
		P3	0 40	–	1 06	0 75	4 44	0 54
3 Sanfilippo s syndrome	M 15	P1	0 89	–	2 74	1 50	3 47	0 37
		P3	0 50	–	0 62	0 78	2 53	0 73
4 Sanfilippo s syndrome	F 9	P5	1 02	–	0 20	0 38	–	0 70
		P6	1 79	–	–	–	–	1 01

In the patient with Pompe s disease a profound deficiency of α glucosidase activity at pH 4 3 was evident (Table 2) At pH 5 8 no definite deficiency could be seen

In the other patients tested no clear enzyme deficiencies could be demonstrated It could also be shown that the patient with cystic fibrosis had a normal ion exchange chromatogram of α fucosidase (see below) This experiment was made because of the reports claiming a change of the fucose/sialic acid ratio in tissues from patients with cystic fibrosis (15)

Molecular forms

By isoelectric focusing and ion exchange chromatography at two different pH values different molecular forms were found for α fucosidase α galactosidase α glucosidase and *N* acetyl-α glucosaminidase (Figs 2–6) Only β mannosidase was seemingly homogeneous in the systems studied *N* acetyl β glucosaminidase was studied concomitantly since the molecular forms of this activity are so well known that they can be used as a model for comparison

α fucosidase was inactivated in the isofocusing system Two fractions could be clearly separated by ion exchange chromatography (Figs 3 and 6)

No definite difference in pH optimum was noted between these two fractions Both exhibited a pH optimum around pH 5 5

α galactosidase was not studied in the isofocusing system Heterogeneity was seen after ion exchange chromatography at pH 7 4 (Fig 4)

The activities were retarded No obvious difference in the pH dependency was noted for the different fractions with α galactosidase activity They all showed a pH optimum around pH 4 5 in 1 M acetate or 1 M citrate buffer

The different isoenzymes of *α glucosidase* could not be separated on isofocusing (Fig 2) while DEAE cellulose chromatography gave at least a partial separation (Figs 3 and 5) The neutral activity of α glucosidase seemed to be more basic than the acidic activity of α glucosidase which therefore was eluted after the neutral activity

It could be shown that the portion of α glucosidase activity eluted first had a neutral pH optimum and the portion of the activity eluted last exhibited an acid pH optimum

β mannosidase activity could not be seen

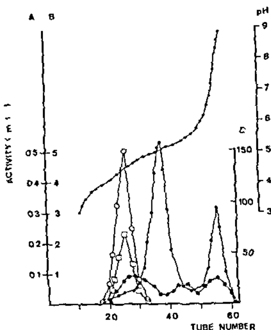


Fig 2 Isoelectric focusing Fibroblast homogenate containing 4 mg of protein was centrifuged at $10^5 \times g$ for 30 min and the supernatant was applied to isoelectric focusing (commercial equipment 440 ml column LKB Produkter Stockholm) The manufacturer s instructions were followed in detail After the carrier ampholytes (pH 3–6 LKB Produkter Stockholm) and the sample had been introduced into the column the electrolysis was started and went on for 30–40 hours at +4°C and at a maximum voltage of 400 V Thereafter fractions of 5 ml were eluted *N*-acetyl β glucosaminidase ●—● α-glucosidase assayed at pH 4 3 □—□ and at pH 5 8 O—O and *N*-acetyl-α glucosaminidase ●—● Scales A B and C refer to α-glucosidase *N* acetyl-α glucosaminidase and *N* acetyl β glucosaminidase respectively Activity is expressed as nmoles substrate cleaved per hour under the above mentioned conditions Assay methods as described in Methods

in the isofocusing system we used (Fig 2) Only one peak of activity was found in the ion exchange chromatography experiments (Fig 4)

N acetyl α-glucosaminidase exhibited heterogeneity both with isoelectric focusing (three peaks Fig 2) and ion exchange chromatography (two peaks Fig 6)

DISCUSSION

The work of Conchie & Hay (6) suggest that *α fucosidase* is a lysosomal enzyme The α fucosidase of ox liver and rat epididymis was studied by Levvy & McAllan (17) The two enzymes were almost indistinguishable The latter authors found a Michaelis Menten constant of about 0 2 mM and a pH-optimum of 5 6 for ox liver and of 6 1 for rat epididymis

Recently Wiederschain et al (24) and Robinson & Thorpe (21) have shown that two forms of α fucosidase exist in human tissues The two forms have different heat stability and molecular weight and thus they separate on gel chromatography They could also be separated on ion exchange chromatography Fucosidase I is a macromolecular form excluded from Sephadex G 200 and adsorbed when ion exchanged on DEAE-cellulose at pH 6 5 The low molecular form called fucosidase II is not absorbed

Durand et al (8) described a disease where α fucosidase activity is completely absent in all tissues examined

Our results using human skin fibroblasts are in good agreement with those of other workers with a Michaelis Menten constant of 0 42 mM and a broad pH-optimum around pH 5 0–6 5 Also two forms could be seen using ion exchange chromatography

Beutler & Kuhl (2) found two types of *α galactosidase* activity (called A and B) in fibroblasts and leucocytes A thermolabile electrophoretically fast component with a low Michaelis Menten constant has been designated as α galactosidase A and a slower thermostable component with a higher Michaelis Menten constant has been called α galactosidase B The residual activity in patients with Fabry s disease contained no detectable A activity only B activity The A form was eluted before the B form when ion exchange on DEAE-cellulose at pH 6 5 was used (3)

Ho et al (12) found optimum activity in fibroblasts at pH 4 5 and a Michaelis Menten constant of 4 mM Isoelectric focusing gave a total of seven peaks the more basic ones were missing in the patients with Fabry s disease

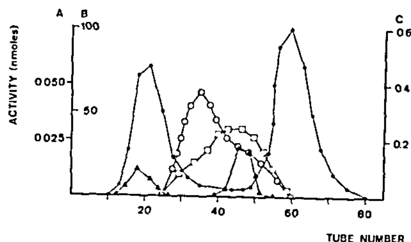


Fig 3 Ion exchange chromatography DE 52 pre swollen DEAE-cellulose (W & R Whatman Balston Maidstone Kent England) was equilibrated in 0 025 M phosphate buffer pH 7 4 A linear gradient was estab lished by 500 ml of phosphate buffer 0 025 M pH 7 4 and 500 ml of the same buffer with 4% NaCl The column was 30×1 5 cm 5 ml fractions were collected Enzyme source was the fibroblast homogenate in water containing 10 mg of protein (6 ml) Before the material was applied to the column it was dialysed against the appropriate buffer *N* acetyl *β*-glucosaminidase ●—● *α* fucosidase ▲—▲ *α* glucosidase assayed at pH 4.3 □—□ and *α* glucosidase assayed at pH 5 8 O—O Scales A B and C refer to *α* glucosidase *N* acetyl *β*-glucosaminidase and *α* fucosidase respectively Activity given as nmoles substrate cleaved per hour Enzyme assay as described in Methods

Our investigation showed a pH optimum at pH 4 5 using 1 M citrate or 1 M acetate buffer The Michaelis Menten constant was 5 0 mM and the DEAE cellulose chromatography showed marked heterogeneity

Several investigations about *α glucosidase* have recently been published using the 4 methylumbelliferyl substrate (7 22) Dreyfus & Alexandre (7) could partly separate the neutral and acidic activity by using cellulose acetate electrophoresis Isofocusing and ion exchange chromatography in our study gave no clear separation of the isoenzyme

Our pH curve found in fibroblast preparations is in good agreement with that of Salafsky & Nadler (22) Our results in a patient with Pompe s disease support the earlier findings that this substrate is very suitable for obtaining diagnosis of acid *α* glucosidase defects (11)

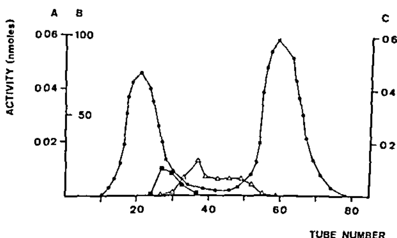


Fig 4 Ion exchange chromatography Experimental conditions and enzyme source was the same as in Fig 3 *N* acetyl *β* glucosaminidase ●—● *α* galactosidase Δ—Δ and *β* mannosidase ■—■ Scales A B and C refer to *α* galactosidase *N* acetyl-*β* glucosaminidase and *β* mannosidase respectively Activity given as nmoles substrate cleaved per hour Enzyme assay as described in Methods

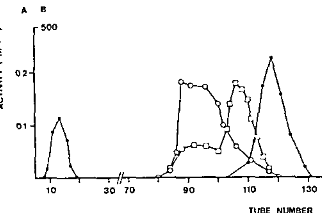


Fig 5 Ion exchange chromatography DE 5? pre swollen DEAE-cellulose (W & R Whatman Balston Maid tone Kent England) The results were obtained by using a smaller column (?4×0 9 cm) The linear gradient was established after 60 ml of phosphate buffer 0 0?5 M pH 6 0 had been put through by 100 ml phosphate buffer 0 0 5 M pH 6 0 and 100 ml of the same buffer with 4 NaCl 1 ml fractions were collected Ultracentrifuged supernatant of a crude fibroblast homogenate in water (30 mg proteins in 6 ml homogenate) was used as enzyme source Before the material was applied to the column it was dialysed against the appropriate buffer *N* acetyl β-glucosaminidase ●—● α glucosidase assayed at pH 4 0 □—□ and α-glucosidase assayed at pH 6 0 ○—○ Scales A and B refer to α glucosidase and *N* acetyl β-glucosaminidase respectively Activity expressed as nmoles substrate cleaved per hour

Muramatsu & Egami (19) describe a β *mannosidase* activity from the liver of *Turbo cortunus* It had a pH optimum about 4 0 and was somewhat activated by sodium chloride The Michaelis Menten constant for the substrate was 7 1 mM The enzyme could be stored several months at −20°C without loss of activity

Properties of β mannosidase in human synovial fluid have recently been studied (1) The pH-optimum was around 3 5–4 0 and the Michaelis Menten constant around 3 4 mM

The β mannosidase in human fibroblasts in our study had a pH optimum at pH 4 5 and the Michaelis Menten constant was 1 7 mM This enzyme differs from the others studied in that it was not possible to dissolve the enzyme into several forms using DEAE cellulose or isoelectric focusing

Weismann et al (23) investigated *N acetyl-α glucosaminidase* from pig liver and showed a pH optimum of 4 6–4 7 The situation was complicated by the unstability of the enzyme below pH 5 when it became insoluble possibly due to aggregation The effect was somewhat reduced by the presence of substrate The authors also found that the variation of homogenate dilution gave a non linear activity which they attributed to endogenous inhibitors precipitable

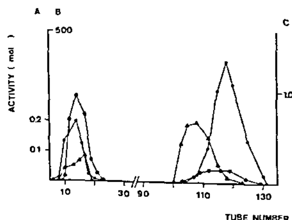


Fig 6 Ion exchange chromatography Experimental conditions and enzyme source was the same as in Fig 5 *N* acetyl β glucosaminidase ●—● α fucosidase ▲—▲ and *N* acetyl-α glucosaminidase ●—● Scales A B and C refer to α fucosidase *N* acetyl-β glucosaminidase and *N* acetyl-α-glucosaminidase Activity expressed as in Fig 5 and the enzyme assay as described in Methods

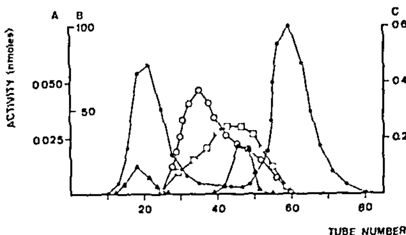


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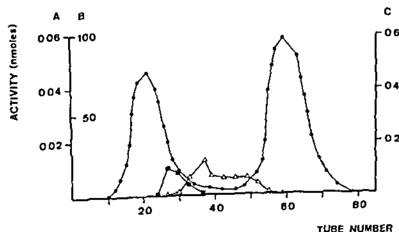


Fig 4 Ion exchange chromatography Experimental conditions and enzyme source was the same as in Fig 3 *N* acetyl β-glucosaminidase ●—● α galactosidase △—△ and β mannosidase ■—■ Scales A B and C refer to α galactosidase *N* acetyl-β glucosaminidase and β mannosidase respectively Activity given nmoles substrate cleaved per hour Enzyme assay described in Methods

21 Robinson D & Thorpe R Human liver α L-fucosidases *Clin Chim Acta 47* 403 1973
22 Salafsky I S & Nadler H L A fluorometric assay of alpha glucosidase and its application in the study of Pompe s disease *J Lab clin Med 81* 450 1973
23 Weismann B Rowin G Marshall J & Friederici D Mammalian α acetylglucosaminidase Enzymic properties tissue distribution and intracellular localization *Biochemistry 6* 207 1967
24 Wiederschain G Y Kolibaba L G & Rosenfeld E L Human α L fucosidases *Clin Chim Acta 46* 305 1973
25 Borjeson M Heinegård D & Öckerman P A Mucopolysackaridos typ III (Sanfilippo s syndrom) *Nordisk Medicin* 81 567 1969

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with ammonium sulphate Michaelis Menten s constant for the phenyl substrate used was 1 4 mM

An N acetyl-α glucosaminidase of ox liver has similarly been reported by Langley & Jevons (16) to show maximum stability at pH 4 5–5 0 with a pH optimum at pH 4 0 These authors found a Michaelis Menten constant of 0 2 mM They could also separate an enzyme preparation into three peaks of N acetyl-α glucosaminidase with fractionation on DEAE cellulose

Recently O Brien (20) and von Figura & Kresse (9) have reported the defect of N acetyl-α glucosaminidase in Sanfilippo type B patients O Brien found the pH optimum in liver and fibroblast preparations to be at pH 4 5 and he also found a non linear response to variation of the homogenate dilution von Figura & Kresse could demonstrate heterogeneity of the enzyme also in human urine Both gel filtration and fractionation on carboxy methyl cellulose gave separation of the different molecular forms

Thus our results are in good agreement with those mentioned above In our study of human N acetyl-α glucosaminidase in fibroblasts we found a pH optimum around pH 4 5 and a Michaelis Menten constant of 0 44 mM Also the heterogeneity could be verified

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REFERENCES

1 Bartholomew B A & Perry A L The properties of synovial fluid β mannosidase activity *Biochim Biophys Acta 315* 123 1973
2 Beutler E & Kuhl W Biochemical and electrophoretic studies of α galactosidase in normal man in patients with Fabry s disease and in Equidae *Amer J Hum Genet 24* 237 1972
3 — Purification and properties of human α galactosidases *J Biol Chem 247* 7195 1972
4 Bosmann H B Identification purification and characteristics of glycosidases of human blood platelets *Biochim Biophys Acta 258* 265 1972
5 Brady R O Gal A E Bradley R M Mårtensson E Warshaw A L & Laster L Enzymatic defect in Fabry s disease Ceramidetrihexosidase deficiency *N Engl J Med 276* 1163 1967
6 Conchie J & Hay A J Mammalian glycosidases 4 The intracellular localization of β galactosidase α mannosidase β N acetyl-glucosaminidase and α fucosidase in mammalian tissues *Biochem J 87* 354 1963
7 Dreyfus J C & Alexandre Y Electrophoretic characterization of acidic and neutral amylo-1 4-glucosidase (acid maltase) in human tissues and evidence for two electrophoretic variants in acid maltase deficiency *Biochem Biophys Res Commun 48* 914 1972
8 Durand P Borrone C & Della Cella G Fucosidosis *J Pediatr 75* 665 1969
9 von Figura K & Kresse H The Sanfilippo B corrective factor A N acetyl-α D glucosaminidase *Biochem Biophys Res Commun 48* 262 1972
10 Hayflick L The limited in vitro life time of human diploid cell strains *Exp Cell Res 37* 614 1965
11 Hers H G α glucosidase deficiency in generalized glycogen storage disease (Pompe s disease) *Biochem J 86* 11 1963
12 Ho M W Beutler S Tennant L & O Brien J S Fabry s disease Evidence for a physically altered α galactosidase *Amer J Hum Genet 24* 256 1972
13 Hultberg B Sjöblad S & Öckerman P A Properties of five acid hydrolases in human skin fibroblast cultures Possible use in the diagnosis of inborn lysosomal diseases *Acta Paediatr Scand 62* 474 1973
14 Kint J A Fabry s disease alpha galactosidase deficiency *Science 167* 1268 1970
15 Kollberg R Lundblad A & Wessler E Urinary excretion of hexosamine sialic acid fucose and acid mucopolysaccharides in healthy children and in children with cystic fibrosis *First Annual Meeting European Working Group for Cystic Fibrosis Stockholm* 1970 p 16
16 Langley T J & Jevons F R Charaterization of beef liver glycosidases possibly involved in glycoprotein degradation *Arch Biochem Biophys 128* 312 1968
17 Levvy G A & McAllan A Mammalian fucosidases 2 α L-fucosidase *Biochem J 80 435 1961*
18 Lowry O H Rosebrough N J Farr A L & Randall R J Protein measurement with the Folin phenol reagent *J Biol Chem 193* 265 1951
19 Muramatsu T & Egami F α mannosidase and β mannosidase from the liver of *Turbo cortunus* Purification properties and application in carbohydrate research *J Biochem 62* 700 1967
20 O Brien J S Sanfilippo syndrome Profound deficiency of alpha acetylglucosaminidase activity in organs and skin fibroblast from type B patients *Proc Nat Acad Sci USA 69* 1720 1972

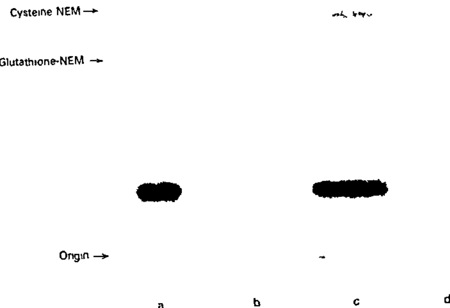


Fig 1 The incorporation of ^{35}S-cystine into leucocytes Autoradiographs after TLC separation of non protein ^{35}S-labelled compounds (*a*) S-cystine marker (*b*) (*d*) extracts of normal leucocytes (*c*) extract of cystinotic leucocytes

pellet obtained by centrifugation at 500 g for 10 min was resuspended in 0 3 ml of Hanks BSS buffered to pH 7 2 with 10 mM Hepes The suspension was mixed with 0 3 ml of ^{35}S-cystine solution (0 05 μmole cystine specific activity 120–200 μCi/μmole Amersham) and 0 3 ml of buffered Hanks BSS (×2 concentration) in a 5 ml glass stoppered conical flask After incubation for 90 min in a shaking water bath at 37 C the incubation mixture was transferred by washing with 5 ml ice-cold 0 9% NaCl solution to a conical glass tube and centrifuged at 500 g for 10 min The cell pellet was washed and centrifuged three times with 7 ml ice-cold 0 9% NaCl solution and then mixed with 50 μl of 1 mM *N* ethylmaleimide (NEM) adjusted to pH 6 4 with NaOH The mixture was frozen and thawed twice and deproteinised by the addition of 10 μl of 3% (w/v) sulphosalicylic acid The precipitated protein was removed by centrifugation and the supernatant analysed for ^{35}S compounds by thin layer chromatography and autoradiography (4)

The gross excess of cystine in the leucocytes of patients with cystinosis is readily detected on visual examination of the developed autoradiographic film Autoradiography overnight is usually sufficient to obtain clear differentiation of cystinotic and normal specimens A typical result is shown in Fig 1 The test should be carried out on fresh blood much of the cystine content of cystinotic leucocytes is lost on storage of specimens for 24 hours at 2 C One or more control specimens should be assayed simultaneously

REFERENCES

1 Schneider J A Bradley K & Seegmiller J E Increased cystine in leucocytes from individuals homozygous and heterozygous for cystinosis *Science* *157* 1321 1967

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SHORT COMMUNICATION

BIOCHEMICAL DIAGNOSIS OF CYSTINOSIS USING LEUCOCYTES

P WILLCOX and A D PATRICK

From the Institute of Child Health London England

ABSTRACT Willcox P and Patrick A D (Institute of Child Health London England) Biochemical Diagnosis of Cystinosis using leucocytes Acta Paediatr Scand 64 132 1975 —A simple method for the biochemical diagnosis of cystinosis involves incubating leucocytes from a small volume of blood with ^{35}S-cystine Non protein ^{35}S labelled compounds are then extracted from the leucocytes and separated by thin layer chromatography The excessive incorporation of ^{35}S-cystine into the increased cystine pool of cystine cells is easily detected in autoradiographs of the chromatograms

KEY WORDS Cystinosis leucocytes biochemical diagnosis

The diagnosis of cystinosis is usually confirmed by examination of the cornea and bone marrow for crystalline deposits However adequate examination of the eye may be difficult without anaesthesia in an infant or uncooperative child and it is our experience that findings for bone marrow are sometimes reported to be negative even for older children in whom cystine crystals would be expected to be abundant Cystine crystals are rarely seen in leucocyte preparations from cystinotic patients and have not been found in their cultured skin fibroblasts Nevertheless the levels of free non protein cystine in these cells are about 100 times the normal (1 2) Estimation of the intracellular concentration of cystine rather than its visualisation as crystals thus provides a more reliable means of biochemical diagnosis that can be applied in the earliest stages of the disease The method has been used recently for the successful antenatal detection of an affected foetus (3) Measurement of the absolute levels of free cystine in cystinotic cells is difficult and requires expensive equipment but for diagnostic purposes it is only necessary to detect the gross excess of cystine in affected cells This can be achieved by a simple inexpensive method involving the uptake and incorporation of ^{35}S cystine into separated leucocytes followed by the extraction of non protein ^{35}S labelled compounds and their separation by thin layer chromatography

All glassware should be siliconised Fresh heparinised blood (1 ml) was mixed with 2 ml of 3% dextran (Dextraven 150) in 0 9% NaCl solution and the erythrocytes were allowed to sediment at room temperature It is convenient to carry out the sedimentation in a 2 ml disposable syringe (total capacity approx 3 ml) placed vertically with the needle uppermost and bent through an angle of 120° After 20–30 min the leucocyte rich supernatant was ejected through the needle and centrifuged at 500 g for 10 min Contaminating erythrocytes in the leucocyte pellet were lysed at 0°C by the addition of water (1 ml) followed after 75 sec by 0 5 ml of 2 7% (w/v) NaCl solution The leucocyte

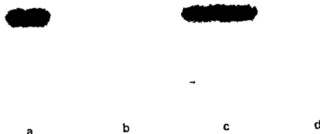


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REFERENCES

1 Schneider J A Bradley K & Seegmiller J E Increased cystine in leucocytes from individuals homozygous and heterozygous for cystinosis *Science* 157 1321 1967

2 Schneider J A Rosenbloom F M Bradley K H & Seegmiller J E Increased free-cystine content of fibroblasts cultured from patients with cystinosis *Biochem Biophys Res Commun* 29 527 1967
3 Schneider J A Verroust F M Kroll W A Garvin A J Horger F O Wong V G Spear G S Jacobson C Pellett O L & Becker F L A Prenatal diagnosis of cystinosis *N Engl J Med* 290 878 1974
4 Willcox P & Patrick A D Biochemical diagnosis of cystinosis using cultured cells *Arch Dis Child* 49 209 1974

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CASE REPORT

SUCCESSFULLY TREATED PNEUMOPERICARDIUM IN A NEWBORN INFANT DURING IPPV

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ABSTRACT Cegrell L and Svenningsen N W (Department of Paediatrics University Hospital Lund Sweden) Successfully treated pneumopericardium in a newborn infant during IPPV Acta Paediatr Scand 64 135 1975.—The successful treatment of pneumopericardium and cardiac tamponade in a newborn infant is presented in this case report Alert recognition of this complication in newborn infants on intermittent positive pressure ventilation (IPPV) is of the utmost importance as prompt and adequate treatment is lifesaving

KEY WORDS Neonate pneumopericardium IPPV

Pneumopericardium has been described in the literature as a rare disease among neonates (cf 11 13) However with the increasing use of artificial ventilation in the neonatal period an increased incidence can be expected as pneumopericardium in most cases has been a complication of intermittent positive pressure ventilation (IPPV) in babies with idiopathic respiratory distress syndrome (IRDS)

Most cases of pneumopericardium reported in the literature did not survive (Table 1) Yet awareness of the possibility of this serious and life threatening complication in infants on IPPV should increase the survival rate as shown by the following case report

CASE HISTORY

771706 *girl infant AS3* birth weight 2150 g was born in the 36th gestational week by Cesarean section as her mother suffered from bronchial asthma

Soon after birth the baby developed *symptoms* of *IRDS* and became apnoic at 8 hours of age She was intubated and artificially ventilated At 11 hours of age she was admitted to the Neonatal Intensive Care Unit of the University Hospital in Lund Chest X ray showed reticulogranular pattern atelectases and bronchogram IPPV with a Servo-900 Ventilator (Elema Schonander Stockholm) was started at once because of persisting severe respiratory insufficiency The respirator settings were initially as follows intermittent positive pressure of 40 cm H_2O and positive end expiratory pressure (PEEP) of 4 cm H_2O The respiratory frequency varied between 35 and 40 per minute according to the blood gas values Antibiotics were given but repeated bacterial cultures of blood and tracheal secretion were negative

During the first hours of IPPV the clinical condition and the blood gas values improved considerably but at 24 hours of age the condition deteriorated with recurring cyanosis Chest X ray revealed pneumothorax in the left pleural cavity and also a pneumopericardium (Fig 1) Pleural drainage was performed with immediate improvement of the clinical condition and at repeat X ray a few hours later the pneumothorax and the pneumopericardium had diminished

During the following days IPPV and bilateral pleural drainage were continued concomitantly On the 5th day of *life* acute deterioration occurred In spite of an increased pleural suction pressure the general cyanosis worsened and the heart sounds became *faint Bradycardia* and finally cardiac arrest followed *Consequently* percutaneous pericardial puncture was performed and simultaneously cardiac massage was started About 20 ml of air was aspirated Instantly a dramatic *improvement*



Fig 1 Chest roentgenograms frontal projection (*a*) and lateral projection (*b*) at the age of 24 hours reveal pneumothorax in the left pleural cavity and a large pneumopericardium

followed with femoral pulses becoming palpable again and cyanosis *rapidly disappearing*

Pleural drainage was carried on for 7 days At 8 days of age the baby was extubated and weaned off the ventilator by continuous positive airway pressure (CPAP) applied with a face chamber (1) for 3 days From 11 days of age artificial ventilation support was no longer required

Control examinations including chest X ray ECG EEG as well as neurological and ophthalmological examinations have revealed nothing abnormal Quantitative plasma immunoglobulins and α_1 antitrypsin were normal At 7 weeks of age the baby was discharged from the neonatal unit weighing 3 090 g At follow up *examinations in the outpatient department* chest X ray has been normal and no signs of neurological sequelae (latest examination at 13 months of age) have been observed

DISCUSSION

The etiology of pneumomediastinum and pneumothorax is considered to be alveolar rupture or rupture of emphysematous blebs or tracheobronchial tear consequent upon endotracheal intubation or too vigorous tracheobronchial suction Air dissects through the interstitium and vascular sheaths into the mediastinum or the pleural cavity (2)

The etiology of pneumopericardium is more uncertain Pneumopericardium is apparently not the result of pericardial tear as this could not be demonstrated by post mortem examination in previously reported cases (7) Most probably air dissects from the interstitium of the mediastinum through the pericardial reflection along the great vessels (9)

The *clinical signs* of pneumopericardium with threatening cardiac tamponade are *cyanosis* and *faint heart sounds* Chest X ray *including lateral projection confirms* the diagnosis It should be noticed that auscultation

Table 1 *Outcome of pneumopericardium in new born infants*

N	IPPV	Survived	Ref
1	–	0	3
1	1	0	4
1	1	0	5
1	–	1	6
3	2	0	7
1	1	0	8
3	3	0	9
1	1	1	10
8	N A	0	11
3	3	0	12
1	1	0	13
2	2	1	14
1	–	0	15
1	1	N A	16
1	1	1	Present report
29		*4*	

N A = No data available

of the heart sounds is an easy and reliable way to diagnose and follow the development of pneumopericardium In the present case we observed that when the pneumopericardium was present the heart sounds became faint or not audible Decreasing pulse rate is an ominous sign and should be considered indicative of cardiac tamponade

Treatment of pneumopericardium should be immediate percutaneous pericardial puncture if cardiac tamponade is suspected When pneumopericardium occurs in combination with pneumothorax it seems advisable to start treatment with pleural drainage if signs of cardiac tamponade are not present If pleural drainage does not substantially improve the clinical condition percutaneous pericardial puncture should be performed immediately

Because of the risk of infection with in dwelling catheters continuous drainage of the pericardial cavity was not used in the present case Some of the cases earlier reported (5 12) survived the critical period with cardiac tamponade but later on died of septicemia Therefore it is important that these patients are treated with antibiotics in adequate doses

ACKNOWLEDGEMENT

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REFERENCES

1 Ahlström H Jonson B & Svenningsen N W Continuous positive airway pressure with a face chamber in early treatment of idiopathic respiratory distress syndrome *Acta Paediatr Scand* 6? 433 1973

2 Avery M E *The lungs and its disorders in the newborn infant* W B Saunders Company Philadelphia and London 1964 p 144

3 Durwar P C Pneumopericardium in neonate *Aust Radio 10* 2?9 1966

4 Gil Rodriguez J A Lewis B W Savege T M Sykes E E & Walling P T Pneumopericardium complicating the treatment of respiratory distress syndrome of the newborn *Br J Anaesth 44* 1?19 197?

5 Grosfeld J L Kilman J W & Frey T R Spontaneous pneumopericardium in newborn infant *J Pediatr 76* 614 1970

6 Kunze J Pneumoperikard nach Spontanpneumothorax in der Neugeborenenperiode *Padiatrik praxis* 9 377 1970

7 Lofus J W Susen A F March J H & Sherman F E Pneumopericardium in infancy *Am J Dis Child 103* 61 196?

8 Lubchenco L Recognition of spontaneous pneumothorax in premature infants *Pediatrics 24* 996 1959

9 Lohrer A Zur Pathogenese des Pneumoperikards beim Neugeborenen *Schweiz Med Wschr 10?* 1248 197?

10 Matthieu J M Nussle D Torrado A & Sadeghi H Pneumopericardium in the newborn *Pediatrics 46* 117 1970

11 Mikity V & Taber P Complications in the treatment of the respiratory distress syndrome *Pediatr Clin N Amer 20* 419 1973

1? Sagel S S Wimbush P & Goldenberg D B Tension pneumopericardium following assisted ventilation in hyaline membrane disease *Radiology 106* 175 1973

13 Shawker T H Dennis J M & Ganes J W Pneumopericardium in the newborn *Am J Roentgenol 116* 514 197?

14 Sigh K R Wigelsworth F W & Stern L Pneumopericardium in the newborn—a complication of respirator management *Canad Med Ass J 106* 1195 197?

15 Weingartner L Pneumoperikard im Neugeborenenalter *Paediatr Paedol 5* ?1? 1969

16 Weller M The roentgenographic course and complication of hyaline membrane disease *Pediatr Clin N Amer 20* 381 1973

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CASE REPORT

WOLFF PARKINSON WHITE SYNDROME

A Fatal Case in a Girl with No Other Heart Disease

A FASTH

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ABSTRACT Fasth A (Department of Paediatrics the General Hospital Helsingborg Sweden) Wolff Parkinson White Syndrome A fatal case in a girl with no other heart disease Acta Paediatr Scand 64 138 1975 — A fatal case of the pre-excitation syndrome in a $3\frac{10}{12}$ years old girl with no other heart disease is presented Her death is thought to be the consequence of ventricular fibrillation This observation is in contrast to the reported benign course in infants and children with the Wolff Parkinson White syndrome without other heart disease

KEY WORDS Wolff Parkinson White syndrome sudden death

The combination of short PR interval and prolonged QRS complex in association with paroxysmal tachycardia constitutes the pre-excitation syndrome first described 1930 by Wolff Parkinson & White (9) Recent advances in the field of electrophysiology with atrial stimulation and His bundle recordings favour the hypothesis that sees the pre excitation beat as a fusion complex between a part of the ventricle prematurely activated through an anomalous pathway and the remaining parts activated via the normal way (6)

Several reports and reviews of this syndrome occurring in infants and children have appeared (2 4 7) They all state that the prognosis in childhood and adolescence is good Sudden death among children seems to be very rare

CASE REPORT

A $3\frac{10}{12}$ years old girl was brought to hospital with no signs of life About 20 minutes earlier when playing she complained of abdominal pains and a couple of minutes later she suddenly lost consciousness Resuscitation was unsucessful

The girl was the only child of healthy parents Pregnancy was uneventful After an uncomplicated delivery in breech presentation bouts of cyanosis were observed when the girl was fed or when crying The electrocardiogram (Fig 1) was typical of a pre excitation type B A roentgenogram of the chest was normal The girl spontaneously recovered during the first days of life and was dismissed without any therapy At every visit the girl was in good health with no cardiac symptoms The electrocardiographic recordings remained unchanged

A few weeks before death the girl complained of brief intense attacks of abdominal pain and during the last week she slept uneasily and was noticeably sweaty at night

Autopsy

The heart was normal in size and shape No congenital cardiovascular defects were observed The myocardium was not thickened There was bilateral pleural effusion as a sign of cardiac strain Microscopical examination showed a slight increase of collagen fibres in the myocardium and scattered degenerated myocardial fibres such as seen in fresh ischemic damage A detailed study of the conductive system was not performed

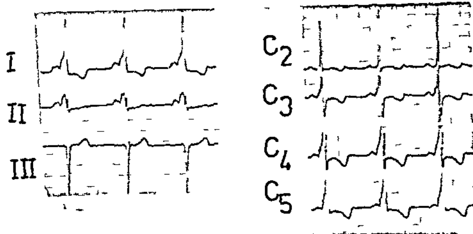


Fig 1 Abnormal electrocardiographic recording showing short PR time delta wave and false left bundle branch block Paper speed 50 mm per sec

DISCUSSION

When sudden death occurs among patients with the pre-excitation syndrome it is generally said to be due to ventricular fibrillation although this has not been demonstrated with accuracy Dreifus et al (3) presented a case where the patient developed ventricular fibrillation during observation of the electrocardiogram on an oscilloscope They reported from the literature six other cases with ventricular fibrillation and the pre-excitation syndrome All the patients were adults and in five of them d c precordial defibrillation was successful In a report of paroxysmal ventricular tachycardia and fibrillation among children by Videbaek et al (8) there are no patients with the pre-excitation syndrome The symptoms before the attack were mostly either non existent or vague such as occasional dizziness and tiredness palpitations or nausea and abdominal pains The symptoms of the girl presented here fit well with death from ventricular fibrillation Her abdominal pain can have been caused by bouts of ventricular tachycardia which terminated spontaneously

Between one and two thirds of the cases with the pre excitation syndrome are associated with a hereditary cardiomyophathy or a congenital heart defect (4 7) A cardiac defect does not seem to increase the risk of a fatal tachyarrhythmia but may of course have more serious repercussions in a child with a cyanotic heart disease At the autopsy of the girl presented here there were no signs of cardiomyopathia and a thorough examination of her parents does not reveal any heart disease

To the accepted therapy with various anti arrhythmic drugs such as *digitalis* and beta blocking agents new possibilities have been added in recent years for prevention of tachyarrhythmias Such are a demand pace maker which by a properly timed impulse terminates the arrhythmia or for a limited number of patients surgical management (1) During the operation the sequence of epicardial excitation is mapped and the anomalous pathway is recognized and transected

As far as the author knows this is the only reported case of Wolff Parkinson White syndrome with fatal outcome in an otherwise healthy child It calls attention to the risk of ventricular tachyarrhythmia which should be borne in mind when examining a child with the pre excitation syndrome and vague symptoms such as tiredness palpitations or

abdominal pains Although the risk of a sudden death is minute these symptoms justify a more careful examination and a close consideration about prophylactic therapy

REFERENCES

1 Cobb F R Blumenschein S D Sealy W C Boineau J P Wagner G S & Wallace A G Successful surgical interruption of the bundle of Kent in a patient with Wolff Parkinson White syndrome *Amer J Cardiol 23* 581 1971
2 Damgård Andersen E Ramsoe Jacobsen J Sandøe E Videbaek J & Wennervald A Paroxysmal tachycardia in infancy and childhood I Paroxysmal supraventricular tachycardia *Acta Paediatr Scand 62* 341 1973
3 Dreifus L S Haiat R Watanabe Y Arriaga J & Reitman N Ventricular fibrillation A possible mechanism of sudden death in patients with Wolff Parkinson White syndrome *Circulation 43* 520 1971
4 Giardina A C V Ehlers K H & Engle M A Wolff Parkinson White syndrome in infants and children A longterm follow up study *Brit Heart J 34* 839 1972
5 Lundberg Å Paroxysmal tachycardia in infancy *Acta Paediatr Scand* Suppl *143* 1963
6 Narula O Wolff Parkinsson White syndrome A review *Circulation 47* 872 1973
7 Swiderski J Lees M H & Nadas A Wolff Parkinson White syndrome in infancy and childhood *Brit Heart J 24* 561 1962
8 Videbaek J Damgård Andersen E Ramsøe Jacobsen J Sandøe E & Wennervold A Paroxysmal tachycardia in infancy and childhood II Paroxysmal ventricular tachycardia and fibrillation *Acta Paediatr Scand 62* 349 1973
9 Wolff L Parkinson J & White P D Bundle branch block with short P R interval in healthy young people prone to paroxysmal tachycardia *Amer Heart J 5* 685 1930

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PROCEEDINGS OF PAEDIATRIC SOCIETIES

EUROPEAN SOCIETY FOR PAEDIATRIC GASTROENTEROLOGY

Seventh Annual Meeting Verona 19–20th April 1974

Gustavsson (Stockholm) *Physiologic role f microflora in the gut in germ free and ex erm free animals*

F K Grutte (Bergholz Rehbrucke) *The nfluence of human milk and different cow s milk formulas on microecological metab olism*

The large number of so called huma nised formulas known today based on cow s milk may be regarded as evidence that the microecological effect of mother s milk has not yet been achieved by any other product In the course of long years of biochemical animal and clinical investi gations on the true causes of the specific putrefaction preventing effect of mother s milk we have found that part of the lactose reaches distal parts of the intestine without being split and absorbed on the way Thereby a specific microbiological biochemical environment is formed which never recurs subsequently In contrast to this lactose in formulas based on cow s milk is split and absorbed before reaching distal parts of the infant intestine We explored the mechanism of these different kinds of lactose metabolism during the passage along the intestine and applied the knowledge so gained to the develop ment of a new simulated mother s milk product

The testing of this product showed posi tive results i e the microecological criteria for this food coincided with those of mother s milk particularly with respect to faecal pH oxidation reduction potential ammonia and bacterial flora including *B bifidus* biotypes

R Bishop (Parkville) *Microflora of stomach and small intestine in acute gastroenteritis*

Recent research on the aetiology of acute gastroenteritis has examined microflora of the levels of gut affected by the disease i e stomach and small intestine

Gastroenteritis in children and adults can be caused by transient colonisation of the upper small intestine by *E coli* strains producing enterotoxin However this is an uncommon cause of sporadic gastro-enteritis in children in Melbourne

The most common aetiology in Melbourne at present is a newly discovered virus of the orbivirus group This neovirus like agent has not yet been grown in vitro Depression of lactase activity in duodenal mucosa during acute gastroenteritis is as sociated with abnormally abundant growth of *Candida albicans* in stomach and duo denum The yeast possesses the ability to depress lactase activity in gut mucosa of infant rabbits It is suspected that its growth in infant human gut might explain develop ment of secondary sugar intolerance after acute gastroenteritis

K Juntunen J K Visakorpi P Kuitunen & E Savilahti (Helsinki) *Intestinal micro flora of children with diarrhoea and mal absorption syndrome*

The possible pathogenetic role of the small bowel microflora in the common chronic intestinal disorders was studied in the following patient groups (i) 15 children with chronic diarrhoea without malabsorption (ii) 12 children with chronic diarrhoea and non specific malabsorption (iii) 8 children with chronic diarrhoea malabsorption syndrome and cow s milk intolerance (iv) 14 children with coeliac disease

The contaminated small bowel syndrome was noted in 40% of patients with chronic diarrhoea in 60% of patients with nonspecific malabsorption syndrome and in 40% of patients with cow s milk intolerance

In coeliac disease the duodenojejunal microflora was similar to that of the controls

The total amount of aerobes and anaerobes did not differ significantly in these groups The aerobic gram negative rods in the duodenojejunal fluid were increased in diarrhoeal patients with or without malabsorption and in cow s milk intolerant patients In the patients with nonspecific malabsorption the duodenojejunal colonisation was most obvious In cow s milk intolerant patients the duodenojejunal colonisation was very similar to that of the diarrhoeal patients without malabsorption syndrome In the faecal microflora no significant differences in these groups were noted

R Nelson (Birmingham) *Small intestinal bacteriology and bile acid metabolism in infants with chronic diarrhoea*

In recent years alteration of the quality and quantity of the bacterial flora of the upper small intestine has been shown to be of considerable importance in relation to the intraluminal phase of digestion and to the absorptive capacity of the mucosa particularly when there is deconjugation of bile acids by anaerobic organisms Significant deconjugation of bile acids only occurs when the bacterial proliferation is of considerable degree as in the blind loop type of situation

Studies in our department by Challacombe et al on infants with chronic non specific gastroenteritis and subsequently by myself on infants with intractable diarrhoea requiring intravenous alimentation have demonstrated that many such infants have bacterial contamination of the upper gut These findings are of unknown significance as anaerobic organisms and deconjugated bile acids were rarely present

Two malnourished infants with intractable diarrhoea had impaired bile acid secretion into the duodenum associated with abnormally high serum bile acid concentrations It is likely that these findings in addition to the pancreatic insufficiency and enteropathy that were found in these patients were related to their severe malnutrition Bile acid metabolism was normal six months later

M Shiner J Ballard & B S Drasar (London) *The influence of gastric pH intestinal stasis immune deficiency and mucosal inflammation on the microflora of the gut*

The upper part of the small intestine is remarkable for its lack of a permanent luminal microflora in healthy subjects Any colonisation of this region with non pathogenic organisms has a profound effect on the absorption of proteins fats and even carbohydrates

Amongst the factors leading to colonisation are achlorhydria and slowing of motility in the small bowel Our investigations have shown a permanent flora within the small bowel lumen in about 80% of subjects with achlorhydria Colonisation in the blind loop syndrome was considerably higher when achlorhydria was also present Apart from

hlorhydria and stasis the immunological ompetence of the bowel lumen and wall ears a relationship to the numbers and ctivity of the bacteria We have investigated e microflora in patients with immune eficiency disease and their immunological elationship to the intestinal mucosa when he mucosal barrier is broken down as in lcerative colitis (U C) In this condition ve have shown that

1 rectal mucosal antibodies in ulcerative olitis react with antigens present on the urface of anaerobes isolated from the atients faeces

2 the reacting antibody is mainly IgG

3 the rectal mucosa in U C contains an ex cess of IgG producing plasma cells

4 an increase in rectal mucosal IgG and complement can be demonstrated by immuno fluorescence in U C

G L Barnes R F Bishop G P Davidson I H Holmes B Ruck & R R W Townley (Parkville) *Histological and ultrastructural abnormalities in acute gastroenteritis of young children*

Fifty two children with acute gastroenteritis were studied by biopsy of the small bowel mucosa All children were less than 4 years old and presented with fever vomiting and diarrhoea of less than 10 days duration Histological alterations of varying degrees of severity were apparent in all but 5 of the children Nine children with histological change initially had repeat biopsy performed and the histological appearance had im proved in each In general depressed di saccharidase levels were found in association with histological damage

Bacterial pathogens could be identified in only 2 of 52 children The presence of histological damage in many children with no recognised pathogen prompted a study of biopsy specimens by electron microscopy Biopsy specimens from 11 of 16 children studied contained virus particles in the cytoplasm of epithelial cells

Three patients in whom virus particles were present underwent a repeat biopsy after recovery from their illness In no instance could particles be seen in the second biopsy specimen

G Benoni E Minelli Bertazzoni T Berti A Deganello G Zoppi & D Gaburro (Verona) *Qualitative and quantitative com position of the faecal flora of neonates fed different milks*

A new technique for evaluating qualitatively and quantitatively the composition of faecal flora has been set up

Using selective media the following or ganisms have been studied

(*a*) aerobes enterobacteriaceae (lactose non fermenting bacteria lactose fermenting bacteria coliforms) enterococci lactoba cilli

(*b*) anaerobes bacteroides enterococci lactobacilli

Faeces of 83 healthy neonates fed breast milk humanized milk formula and partly skimmed edulcorated cow s milk have been examined at birth and at the second and fifth day of life

The preliminary results seem to indicate that the most important differences during the first week of life between the faecal flora of neonates fed different diets consist of

1 higher total bacterial count (aerobic and anaerobic) in faeces of neonates fed cow s milk (humanized and partly skimmed edulcorated)

2 no statistically significant differences between the two types of cow s milk used

A S McNeish C J Rolles & R A Thompson (Birmingham) *Evidence of com plement activation after gluten challenge in treated coeliac children*

There is accumulating evidence that an antigen antibody reaction may be involved in

the pathogenesis of coeliac disease (CD) which may result in complement activation Eighteen coeliac children who had been on a gluten free diet for 6 months to 8 years were given 20 g of oral gluten and plasma samples were assayed at 0 4 8 24 hours for Clq C4 C3 C6 C7 and for the presence of C3 breakdown products (α_2D) The diagnosis of CD was subsequently confirmed in 13 cases when by continuing oral gluten for 1–6 months a malabsorptive state with villous atrophy was induced Three children had no symptoms after 1–2 years of gluten ingestion and the jejunal histology remains normal in each case they are considered not to have CD Two children have normal jejunal histology after 4–8 weeks of gluten reintroduction though both have had gastrointestinal symptoms suggesting CD the diagnosis is considered to be equivocal

Of the 13 proven coeliacs 12 had a positive gluten challenge as measured by falling complement levels and the appearance in the plasma of C3 breakdown products at 8–24 hours The 3 non coeliacs had negative results Of the 2 equivocal children one showed reduced complement levels and the other had normal results throughout the test

Assessment of complement activation after gluten challenge may increase understanding of the pathogenesis of CD and may aid diagnosis in difficult cases

B McNicholl B Egan Mitchell & P F Fottrell (Galway) *Varying gluten susceptibility in coeliac disease*

Of 208 children with coeliac disease 40 were given a trial of normal diet at a mean age of 9 7 years they had been on gluten free diets for a mean of 6 years and the duodeno jejunal mucosal histology had returned to normal in 39 in one no biopsy was available (Mucosal changes 0=normal I=slight II=moderate III=severe)

In 36 children mucosa reverted to G II or III in a mean of 15 4 months (4 to 35 months) In 6 symptoms appeared and g III mucosa was found in 4 to 14 months 3 of these showing a fall of 5 or more length centiles and one of them a low serum folate 15 children had no symptoms but showed falls of 5 or more length centiles 4 having low folate and 2 low serum iron Four children without symptoms or growth impairment but haematology was incomplete

Four children still have G 0 mucosa 20 to 52 months after return to a normal diet and all have shown accelerated growth and normal haematology except one with low iron Their mean mucosal lactase before gluten challenge was 67 units the final mean lactase being 18 units just more than 1 S D below control means

Clearly 36 children have permanent gluten intolerance but there has been considerable delay in reversion to a flat mucosa in some We believe that the 4 children with morphologically normal mucosa may have latent coeliac disease and will revert to a flat mucosa with continued gluten ingestion or with major stress

S Packer R J Rowlatt & J T Harries (London) *Reappraisal of a past diagnosis of coeliac disease*

In true coeliac disease intolerance to dietary gluten is permanent 1 in 10 first degree relatives are affected and there may be an increased risk of neoplasia The implications to the child and its family are major and the application of strict diagnostic criteria by means of intestinal biopsy is essential

This paper presents the results of a study designed to reassess a past diagnosis of coeliac disease in 44 patients aged 4–16 years The patients fell into 1 of 3 groups *Group I* had received a gluten free diet (GFD) for 1–13 (mean 6) years with no past biopsy (19) or past biopsy considered uncharacteristic on review (5)

Group 2 had received a normal diet (ND) for ½–6 (mean 2½) years following a GFD for ½–5½ (mean 2¾) years with no past biopsy (12) or uncharacteristic biopsy on review (2) *Group 3* ND for 2½–9¾ (mean 6) years following GFD for 2–5½ (mean 3½) years with past biopsy characteristic (6)

Biopsies were performed in Group 1 before and after gluten challenge (>10 g wheat protein per day for 3 months) Challenge induced mucosal abnormalities in 18 (75%) and of these only 7 (39%) developed symptoms 5 (38%) patients in Group 2 were proved to have coeliac disease 3 (50%) patients in Group 3 had normal biopsies (transient gluten intolerance?) and the remaining 3 patients had flat biopsies despite normal growth and RBC folate levels

These results illustrate the importance of applying strict diagnostic criteria to patients with suspected coeliac disease and the necessity of reassessing a past diagnosis of coeliac disease

P Rodesch S Cadranel M Winkler & H Loeb (Brussels) *Hypovitaminosis K in coeliac disease*

Hypovitaminosis K is well known in infants fed with maternal milk and also in cases of acute diarrhoea treated with antibiotics Vitamin K deficiency has also been reported in malabsorption syndromes such as adult coeliac disease and cystic fibrosis recently hypovitaminosis K has been described in a child with marasmus probably due to coeliac disease

We report the case of a nine month old female infant who presented with bruising as main symptom After several months on a low fat diet for frequent greasy stools she has been on antibiotics for an acute diarrhoea 3 weeks prior to admission Laboratory data showed prolonged prothrombin time (less than 10%) which was corrected within a few hours after vitamin K given parenterally

A malabsorption syndrome could be demonstrated and the jejunal biopsy showed subtotal villous atrophy

After 3 months on cow s milk and gluten free diet clinical biological and also histological improvement could be observed

On subsequent non observance of the gluten free diet clinical and histological relapse occurred

This case points out the importance of the three major factors (deficient intake malabsorption and treatment with antibiotics) predisposing to hypovitaminosis K

M Cribbin J A Walker Smith & C B S Wood (London) *Gastroenteritis and its sequelae A prospective review of experience in a children s hospital*

Acute gastroenteritis in childhood is still a serious problem in developed as well as in developing countries In 1973 admissions to the gastroenteritis unit at Queen Elizabeth Hospital for Children in London totalled 472 i e 6% of all the hospital s admissions

A prospective study of the admissions to the gastroenteritis unit in 1973 has shown that 240 admissions were for acute gastroenteritis 126 for acute gastroenteritis accompanied by signs of infection outside the alimentary tract such as upper respiratory tract infection and otitis media 27 admissions for isolation and the remaining admissions proved not to be suffering from gastroenteritis but from a variety of illnesses with symptoms resembling gastroenteritis

Most children were aged less than one year Bacterial pathogens were isolated from the stools in only 71 i e in 11% of the children Four children died in the unit during the course of the year but in only two children was this directly due to gastroenteritis (mortality 0 6%) i e the mortality of gastroenteritis was low

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There was however a much higher level

of morbidity Delayed recovery following gastroenteritis e g recurrent diarrhoea and failure to thrive occurred in 85 children This was due to sugar intolerance in 39 children In 25 children small intestinal biopsy was performed in 13 and abnormal mucosa was shown In the remaining children with delayed recovery no definite cause for this was established However morbidity after gastroenteritis clearly occurred more often in infants under the age of 3 months and also in immigrant children There is a clear need for further investigation of the sequelae of gastroenteritis in childhood

J Navarro C Polonovski J Salet J Fage C Van Kote & J F Mougenot (Paris) *Chronic diarrhoea by vagal excitation after fundoplication without pyloroplasty*

Diarrhoea is a well known complication of Nissen type fundoplication for hiatal hernia The common explanation for this protracted diarrhoea is the usual accompanying pyloroplasty One peculiar observation suggests another explanation for post Nissen diarrhoea

A ten month old child was operated for hiatal hernia with relapsing gastrointestinal bleeding and oesophagitis The operation was Nissen fundoplication but without pyloroplasty and a 10 day temporary gastrostomy Diarrhoea first appeared with peroral alimentation and rapidly became severe For 4 months various diets with milk and gluten exclusion were attempted Diarrhoea was unchanged and the weight curve remained invariably flat There was no evidence of clinical or biological symptoms of intestinal malabsorption, sugar intolerance or cow s milk intolerance There were neither symptoms of hernia recurrence nor of oesophageal stricture following peptic oesophagitis

Hyperperistaltism was established on the following symptoms (i) red carmine staining appearing in stools in one or two hours after oral administration (ii) hyperglycemic curve of dumping syndrome type (iii) hyperkinetic changes of X ray studies with instantaneous jejunal repletion (iv) continuous feeding by gastric tube allowing dramatic recovery with impairment of diarrhoea and spectacular weight gain

Normal gastric acidity and normal gastrinemia eliminated the Zollinger Ellison syndrome The hypothesis of continuous vagal excitation by fundoplication was based on the former symptoms but also on the effects of administered atropine and the possibility with this drug of normal peroral alimentation

Recurrence of clinical biological and radiological symptoms was rapidly obtained with atropine cessation Equilibration occurred with atropine readministration

This observation may demonstrate that pyloroplasty is not necessary for explanation of the post Nissen diarrhoeal syndrome and that vagal excitation may be another pre eminent factor

W Meier Ruge (Basel) *Newer aspects of the histopathology of Hirschsprung s disease and related disorder of the colon*

Hirschsprung s disease is typified by aplasia of the intramural ganglionic cells in the spastic intestinal segment In addition there is extremely high activity of acetylcholinesterase in the extramural parasympathetic nervous system This increased activity can be observed even in tiny pieces of mucosal biopsies and provides a simple and reliable diagnosis of Hirschsprung s disease

The fact that only the distal segment of the colon is affected in Hirschsprung s disease is the result of the special innervation found throughout the distal colon It was possible to show by morphometric measurements that the extrinsic parasympathetic innervation decreases exponentially

from the anal sphincter to the left colonic flexure There is a steep increase in circular muscle innervation in the cranio caudal direction with a ratio of 1 10 It corresponds to the increase of contractile and propulsive force from the splenic flexure to the anal sphincter This provides the explanation for the maximal contractility of the lower rectum in aganglionic disorders On the other hand the density of extrinsic para sympathetic nerve fibres characterizes several spastic or atonic forms of constipa tion because the propulsive activity of the distal colon is controlled by these nerves From the therapeutic point of view it is possible to specifically activate the extrinsic parasympathetic nerves of the distal colon In this way a new pharmacotherapeutic concept for the treatment of constipation of the distal colon e g the atonic megacolon and so on is possible

Besides Hirschsprung s disease two other disorders of the colon must be mentioned (1) Hypoganglionosis of the colon and (2) Neuronal colonic dysplasia

Hypoganglionosis of the colon may occur as an independent disease Often hypoganglionosis follows on the aganglionic Hirschsprung segment This fact was con firmed by morphometric investigations The recognition of the possible existence of a hypoganglionic segment in association with an aganglionic segment in Hirschsprung s disease is of interest in assessing the length of bowel which is to be resected by the surgeon

Neuronal colonic dysplasia has very often a clinical symptomatology like that of Hirschsprung s disease This disorder is characterised by an extreme spasticity of the mucous and myenteric plexus an increased acetylcholinesterase activity in the para sympathetic nerve fibres of the lamina propria and an aplasia or hypoplasia of the sympathetic innervation of the myenteric plexus This insufficiency of sympathetic in hibition of the parasympathetic activity of the nervous plexus in the colonic wall may explain the clinical symptomatology of this colon dis ease As in Hirschsprung s disease the treat ment of neuronal colonic dysplasia (in its ad vanced forms) consists of removing the distal intestinal segment since the latter determines the course of the disease

The development of enzyme histochemical methods for the diagnosis of Hirschsprung s disease have made the histopathology of this colon disorder more simple and reliable Furthermore these new methods have given much greater accuracy in the differential diagnosis of Hirschsprung s dis ease and conditions producing similar signs and symptoms Finally we gain better insight into pathogenesis and pathophysiology of propulsive disorders of the colon

A F Schärli (Luzern) *Surgical aspects of Hirschsprung s and related diseases*

Aganglionic megacolon is a congenital anomaly characterized by partial to complete distal colonic obstruction due to an absence of ganglionic cells in the myenteric plexuses of Auerbach and the submucosal plexus of Meissner within the colonic wall The degree of obstruction and the clinical course vary with the length of the aganglionic segment intercurrent infections enterocol itis and other undefined pathologic factors Hirschsprung s disease can be recognized in newborn and young infants as mild in testinal obstruction associated with ab dominal distention Opaque enemas allow diagnosis with recognition of the site of transition to normally innervated colon Rectal suction biopsy and histochemical examination is mandatory for definite diag nosis

Symptomatic Hirschsprung s disease in children under three months is a potentially fatal disorder requiring prompt colostomy as an emergency procedure before the devel opment of enterocolitis This aggressive approach to the treatment of young infants

is dictated by the high mortality (up to 30%) in untreated infants Children with severe enterocolitis rarely survive

Contrary to many other authors we believe that colostomy in less severe cases is avoidable

The definite treatment of Hirschsprung s disease is surgical excision of the aganglionic colon and rectum with preservation of sphincter control The abdominal and perineal procedure introduced by Swenson is a standard accepted operative procedure However this technique is time consuming and demands attention to detail if serious complications are to be avoided Modifications aimed at simplifying and making the operation safer have been introduced The most frequently used is the Duhamel and the Grob procedure and their various modifications

In recent years the endorectal pullthrough operation known as Soave s procedure and its modifications has been widely accepted All of these operations tend to solve the problem of Hirschsprung s disease though none of them is free from complications

Experiences with sphincterotomy and rectal myotomy through a posterior incision because of short segment Hirschsprung s disease or residual symptoms following Swenson s procedure are quite satisfactory In the event of the aganglionic segment being longer than anticipated the performance of a more extensive procedure is in no way compromised Considering all the important factors of Hirschsprung s disease the mortality in experienced hands can be less than 5%

F M Penninckx & R Kerremans (Leuven) *Evaluation of anorectal motility and recto anal reflex in Hirschsprung s disease and functional constipation*

Anorectal electromanometrical recordings of 30 patients with Hirschsprung s disease (determined by biopsy) 30 children with functional constipation and 20 normal children were analysed The micro balloon technique for pressure recording was used A balloon for air insufflation was placed separately in the rectum to obtain rectal distension

Basal tension in the rectum does not significantly differ from normal suggesting that in Hirschsprung s disease the rectum is not in a state of contracture—in contrast to the general concept Basal tension in the anal canal is within normal limits suggesting that the internal sphincter is not hypertonic in these diseases

Rectal motility is increased in patients with functional constipation and Hirschsprung s disease

Anal motility differs significantly from the normal pattern and specific changes are noted for functional constipation and for Hirschsprung s disease The frequency of the anal slow pressure waves is increased in constipation and diminished in Hirschsprung s disease the amplitude of these waves is normal or slightly increased in functional constipation but strongly increased in Hirschsprung s disease In 33% of our patients with Hirschsprung s disease simultaneous contraction waves of long duration and high amplitude were recorded in the rectum and anal canal These waves were not seen in normal children or patients with functional constipation

In the present series the value of an absent recto anal reflex for the diagnosis of Hirschsprung s disease is 96 8% Failure of the test may be due to pronounced megarectrum in cases of functional constipation However the pattern of the resting motility may be another sign for differential diagnosis

It may be concluded that rectal biopsy can be adequately substituted by anorectal electromanometry and reserved for difficult cases only

H Verder P A Krasilnikoff & Elma Scheibel (Hellerup) *Anal tonometry in mature and premature children*

Only a few investigations of the anal sphincteric reflexes have been carried out in the neonatal period and with conflicting results because of the different techniques used

A new hollow cylindric anal tonometer of small dimensions was therefore constructed Three balloons were used for the measurements one placed in the rectum one corresponded to the internal and one to the external anal sphincter The reflexes were produced by air insufflation into the rectal balloon

With this tonometer a relaxation of the internal anal sphincter after distension of the rectum could be demonstrated on the first or second day of life in both mature and premature babies without intestinal symptoms In some cases this reflex could not be demonstrated until the third day of life due to obstruction of the tonometer by meconium Some of the children showed a spontaneous rhythmic activity in the internal anal sphincter as well These results closely resembled those seen in normal older children and adults

It was furthermore possible in four children between 11 and 46 days of age to demonstrate abnormal sphincteric reflexes similar to those seen in older children with Hirschsprung s disease and aganglionosis was thereafter verified by rectal biopsy

R Lagercrantz & L Billing (Stockholm) *On constipation*

C Ricour & S Balsan (Paris) *Total colonic aganglionosis ileocoloplasty and sodium conservation*

Aganglionosis affecting the entire colon and terminal ileal loops represents one of the most severe forms of Hirschsprung s disease A permanent ileostomy or an ileo-anal anastomosis result in sodium depletion To prevent this complication a lateral anastomosis between the aganglionic descending colon and the normal ileum is recommended associated with a posterior rectal sphincterotomy The aim of this study was to investigate sodium conservation after this kind of surgical approach Balances of water sodium and potassium have been performed during progressive sodium restriction in two infants with ileocoloplasty and the results of these balances were compared with those obtained in five infants deprived of their colon In the absence of the colon an adaptation of the small intestine to conserve sodium is demonstrated but this adaptation was limited since signs of sodium depletion appeared when the intake of sodium was reduced to 1 mEq/kg/24 h On the other hand in infants with ileocoloplasty the reduction of sodium intake to 0 3 mEq/kg/24 h is well tolerated without any sign of sodium depletion The variations in the concentration and the 24 hourly faecal excretion of sodium and potassium are similar to those observed in colostomies

Ileocoloplasty provides good sodium conservation prevents accidents due to sodium depletion and constitutes a satisfactory and physiological approach to the treatment of total colonic aganglionosis

R Kerremans J Beckers F Trimpeneers F Penninckx & E Eggermont (Leuven) *Postoperative complications and long term results of different types of surgical resection in Hirschsprung s disease*

A new combined operation for treatment of Hirschsprung s disease was performed in 15 cases taking into account the high incidence of postoperative complications after Swenson s operations and intrasphincteric resection (61% and 75% respectively) The technique is a combination of the Swenson operation (though a

larger part of the distal rectum is preserved) and of an extra mucosal myotomy of the residual rectum and of the internal sphincter Only in 27% cent of the cases were post operative complications noted

The long term effects on anal function were classified as normal soiling pseudo incontinence and incontinence A trial for objectivity was performed by means of electromanometry and bariumenema Normal anal function was noted in 85% after Swenson s operation and after the combined operation and was characterized by increased anal basal pressure compared with the preoperative state Soilers and patients with pseudoincontinence (respectively 16% and 13% of the postoperative cases) had lower pressures than the normal post operative cases The radiological diameter of the lower colon compared with the diameter of the pelvic inlet (Colo Pelvic index) was smaller than 1/2 in normals and in soilers and larger than 1/2 in patients with pseudoincontinence True faecal incontinence was only noted after intra sphincteric resection

D Nussle N Genton C Bozic M Landry & D Berger (Lausanne and Geneva) *Functional radiological findings in short terminal forms of Hirschsprung s disease and other causes of dyschezia*

Eighty seven cases of dyschezia were studied by a dynamic radiological examination and by a Swenson ano rectal biopsy

For the radiological examination a 70 mm camera and a video tape recorder were used After filling the colon with a barium suspension the behaviour of the rectum and of the anal canal before and during the act of defecation was examined and if possible in the sitting position Biopsy samples included a longitudinal band of the entire width of the rectal wall beginning 2 cm from the ano rectal margin In 20 cases a histochemical stain for acetylcholinesterase was applied A good correlation between this method and conventional histological techniques was found 17 cases of Hirschsprung s disease were examined 14 of them had a short aganglionic segment including a portion of the rectum Post operative studies were also done in 17 cases by Duhamel Soave or Swenson procedures In all of these cases the proximal and anterior part of the anal canal was found unrelaxed and narrowed even during the rectal contraction confirming the lack of relaxation of the internal sphincter in this disease

In the other 53 cases of dyschezia in which the Swenson biopsy indicated a normal intramural plexus the radiological findings could be classified into four different types (1) atonic terminal sac without defecation (8%) (2) failure of *opening of the inferior part* of the anal canal spasm of the external sphincter (30%) or true anal stenosis (17%) (3) hypertonia of the puborectal sling alone (4%) (4) lack of relaxation of the puborectal sling and of the external sphincter (41%) In all of these cases of functional dyschezia with the exception of two cases of type 1 a good *opening of the internal sphincter* which can be seen anteriorly was observed

This functional radiological examination therefore appears to be useful for selection of those cases that may require rectal biopsy and to assess postoperative results

P M Farrel J C Fratantoni J G Bieri and P A di Sant Agnese (Washington) *Effects of vitamin E deficiency in man*

Protein manifestations of tocopherol deficiency occur in animals but man has not been shown to develop clinical symptoms Previous studies have been hampered by the small population of subjects available for investigation Patients with cystic fibrosis (CF) provide the best available model of vitamin E deficiency in the human In order

to evaluate the effects of tocopherol deficiency in man we have focused on some of the main disturbances found in animals as well as performing electron microscopic studies *Plasma and α-tocopherol* (α T) Plasma α T determined after thin layer chromatography was uniformly decreased in 50 patients with pancreatic achylia due to CF (mean = 125 μg/100 ml range = 23–410 control mean = 730 range = 550–960) Erythrocyte α T was decreased in parallel fashion and averaged 42 μg/100 ml of packed red cells in 5 patients as compared with a normal mean of 230±13 μg/100 ml (Bieri & Poukka *Int J Vit Res* *40* 344 1970) Oral supplementation with 3–5 IU/kg/day of water miscible α T resulted in normal plasma levels and in a limited number of cases at autopsy normal concentrations of α T in muscle and various other organs *Hemolysis in vitro* by an improved H_2O_2 technique invariably accompanied vitamin E deficiency and showed a sigmoidal relationship to plasma α T level Determination of ^{51}Cr RBC survival revealed a mean $t_{\frac{1}{2}}$ of 22 days in 19 deficient patients with 3 subjects particularly low at <18 days the average $t_{\frac{1}{2}}$ in 6 CF patients after α T supplementation increased from 19 to 27 days

Muscle involvement Creatinuria was present in 13 of 25 patients studied with the total group of 25 showing an average creatine excretion of 200 mg/24 hr (normal mean = 20 mg/24 hr $p<0.01$) Elevated plasma aldolase and CPK (the skeletal muscle isozyme) have been found in 2 out of 40 patients thus far

Subcellular membraneous structures in intestinal biopsy samples were similar in appearance for control subjects and α T deficient patients

Conclusions In patients with CF there are no major clinical effects of vitamin E deficiency but the above data support the need for α T supplementation These findings can be applied to malnourished populations at large (e g Bangladesh Jordan etc)

A Rubino & B De Vizia (Naples) *Dipeptide transport across brush border of human jejunum*

Previous studies in rabbits have shown that amino acids can be translocated as peptides from intestinal lumen to cell by way of a transport system selective for peptides In the present study dipeptide uptake is measured in biopsy mucosal specimens of human jejunum

Unidirectional influx of Glycyl L Proline (GP) from medium to tissue is determined by exposing the tissue to 0 5 mM GP for 30–60 seconds GP influx (μmoles/g tissue hr) is 10 3±0 9 (Mean±S E M) ($n=17$) while influx of free Glycine (0 5 mM Glycine in the medium) is 2 7±0 5 ($n=5$) Under sodium free conditions GP influx is reduced to 48±10 2% of the control When amino acids or dipeptides are added to the incubation medium at 20 mM concentration GP influx (% of control) is 96 7±5 0 with Glycine 111 1±4 1 with L Proline 76 5±3 3 with L Arginine 111 9±9 9 with L-Glutamic Acid 17 6±0 5 with I Phenylanyl L Alanine 23 4±1 2 with L Leucyl L Proline

If incubation in the presence of 0 5 mM GP lasts 15–60 minutes glycine within the intracellular fluid reaches a concentration exceeding that of GP in the medium The results indicate that glycylproline is translocated across the brush border of human jejunum by a very efficient transport mechanism which is shared by other peptides but has little or no affinity for free amino acids

S P Lamabadusuriya E Guiraldes J Keeling & J T Harries (London) *The protective effects of mixed micellar solutions on deoxycholate induced (I) inhibition of transport (II) structural abnormalities and (III) ATP ase inactivation in the rat jejunum in vivo*

Bacterial overgrowth of the small intestine

occurs in a variety of infantile diarrhoeal states but the relationship between the abnormal flora and the diarrhoea is not clear. Bacterial degradation of bile salts is known to generate toxic products, in particular the unconjugated dihydroxy bile acid deoxycholate (DCA); this bile acid occurs in the small intestinal lumen of patients with diarrhoea and has been implicated in the pathophysiological mechanisms responsible for the diarrhoea.

We have investigated the effects of micellar solutions on the toxic effects of deoxycholate in rat jejunum using both an *in vivo* closed loop and perfusion technique. DCA in concentrations as low as 1 mM inhibited water, electrolyte and glucose absorption and mucosal ATPase activity; structural mucosal abnormalities were induced by concentrations >2.5 mM. These effects persisted when DCA was solubilised in pure taurocholate micelles.

Mixed taurocholate micelles (containing oleic acid, caprylic acid or monolein) were prepared and deoxycholate solubilised in each of the solutions. The mixed micellar solutions produced a highly significant protection against each of the toxic effects of DCA which were studied. Oleic acid emulsions had no protective effect. The rate of disappearance of ^{14}C DCA from the lumen was reduced by solubilisation in mixed micellar solutions but not by solubilisation in pure bile salt micelles.

These results show that mixed micellar solutions protect against the toxic effects of DCA in the jejunum and that the bile salt–lipid complex retards luminal disappearance of DCA; protection may be achieved by solubilisation of DCA within the expanded hydrocarbon core of the fixed micelle. Thus, the clinical effects of DCA in man may be dependent on its physiochemical state in the intestinal lumen, the concentration of conjugated bile salts, the efficiency of pancreatic lipolysis and the dietary intake of fat.

S. W. Bender, F. Michel & B. Hadorn (Frankfurt a. M.): *Effect of bile duct transplantation of intestinal mucosal enzymes in the rat*

The longitudinal distribution of sucrase and enterokinase in the rat small intestine was first established. Enterokinase activity, which is highest in the duodenum, shows a sharp rise at the papilla Vateri, whereas sucrase is low in the duodenum and increases gradually to a maximum in the middle part of the small bowel. The possibility that the pattern of enterokinase distribution is influenced by biliary or pancreatic factors was studied with biliary tract ligation and diversion of the biliary flow to lower segments of the intestine. It was found that the peak of enterokinase activity was always located at the point where the diverted bile flow reached the small intestinal lumen. Pancreatic enzymes did not influence mucosal enterokinase activity. The effect of bile is not due to a change of specific activity of enterokinase molecules present in the mucosa. It is suspected that either the enzymatic synthesis or intracellular distribution of the enzyme is changed under the influence of bile. It is of interest to note that the small intestine is capable of increasing the activity of an enzyme in a location in which it is normally present only in small amounts. In man enterokinase is a duodenal enzyme. The results obtained in the rat may be of importance in explaining why protein digestion is not severely impaired in patients with duodenectomy.

T. Lindberg (Malmo): *Protease inhibitors in human milk*

The presence of trypsin, chymotrypsin, elastase and pepsin inhibitors in human milk from the first day to the fourth month after birth has been studied with a gel diffusion method. Half of the samples from the first day after birth and nearly all the samples

om the second day to the fourth month ntained trypsin chymotrypsin and elas se inhibitors No pepsin inhibitor was und Quantitative studies showed that ml of human milk inhibits 25–50 μg of ovine trypsin Crossed immunoelectro horesis with antisera against human α_1 ntitrypsin and human antichymotrypsin emonstrated the presence of α_1 antitrypsin nd antichymotrypsin in human milk Using ne electroimmuno assay of Laurell these wo inhibitors were quantitatively estimated The highest concentration was found in amples from the first week after birth

E Petrich & W Plenert (Jena) *The concentration of essential fatty acids in human milk*

The composition of human milk lipids is of interest in nutritional physiology with respect to recommendations for artificial diets Analyses of 47 samples of fresh human milk were made by thin layer and gas liquid chromatography There are some characteristic changes of the fatty acid patterns of triglycerides (95% of human milk lipids) during different periods of lactation Most of these variations are not significant but there is one exception The concentration of linoleic acid (C 18 2) rises from 7% on the 3rd day after delivery up to more than 11% during the 10th month of lactation ($0.005 > p > 0.001$) Accepting human milk as a standard for artificial diets it has to be considered that the standard deviation is about 15% meaning that the concentration of linoleic acid in human milk triglycerides shows a variation of between 8 and 13% In cow s milk linoleic acid constitutes 1 5–2 8% of total fatty acids (0 7–1 4% of total calories) In human milk 5–6% of total calories are supplied by linoleic acid Human milk fat contains a small fraction of alkoxydiglycerides (10–30 mg/100 ml milk) Furthermore there are free fatty acids esterified cholesterol (traces) free cholesterol monoglycerides and phospholipids The concentration of total cholesterol is significantly higher in human milk (30–65 mg/100 ml) than in cow s milk (13–15 mg/100 ml)—this difference might give an explanation for the higher concentration of serum cholesterol in breast fed infants versus those fed formula diets

A Ferguson & T T MacDonald (Glasgow) *Activated T lymphocytes in clinical and experimental small bowel disease*

Lymphocytes are a normal component of the small intestinal mucosa In health the majority are probably B lymphocytes (1) but there is some experimental evidence that in conditions associated with villous atrophy much of the small bowel damage may be mediated by activated T lymphocytes (2)

We have measured three lymphocyte activation products (lymphokines) in homogenates of small bowel tissue and in small bowel organ culture fluid The lymphokines studied were macrophage migration inhibitory factor (MIF) skin reactive factor (SRF) and mitogenic factor (MF) Preliminary results indicate that lymphokines can be detected in the small intestine and that their presence may be associated with small bowel disease

B Mougenot J L Fontaine J F Mougenot & C Polonovski (Paris) *Development of intestinal Ig containing plasma cells in normal infants and children*

Plasma-cells containing IgA IgM and IgG have been detected in the lamina propria of normal intestinal mucosa of 47 children aged 0–14 years by direct immunofluorescent technique using specific fluorescein labelled antisera Their number was compared with the serum immunoglobulin levels of these normal infants and children

1 In all duodeno-jejunal biopsy specimens from 42 children aged 3 months to 14 years

a heavy population of immunocytes was observed with a predominance of IgA-containing cells which in most cases outnumbered those producing IgM and IgG However the intestinal IgA cell population was found significantly smaller in infants aged 3–18 months than in children over 18 months

During the different periods of childhood there was a positive correlation between the average number of intestinal IgA cells and the level of serum IgA This could not be evidenced for IgM

2 On the other hand in 5 specimens of small gut or colon from young infants whose ages ranged from 6 hours to 2 months 9 days IgA cells were found to be scarce or very scarce It seems that IgA immunocytes begin to appear in the gut mucosa within the first days or the first weeks of life to become really numerous during the second or the third month

The synthetic mechanisms of intestinal secretory IgA grow and mature earlier than those of serum IgA in normal children

M Forars M Hagstrom E Rasanen & P Kuitunen (Helsinki) *Recurrent abdominal pain in children aged 6–12 years*

Seventy five consecutive patients aged 6–12 years with recurrent abdominal pain were examined at the Children s Hospital University of Helsinki Finland All children were examined in the same way and by the same psychologist child psychiatrist social worker and pediatrician In addition to routine examinations lactase tolerance test (with ethanol) pentagastrin test X ray examination of the stomach and small bowel and in some cases i v urography were also performed

A somatic cause was found in only 8 children (5%) An abnormal lactose tolerance test was controlled by determination of the lactase enzyme content in a proximal jejunal biopsy specimen It was abnormal in one and borderline in one case The variations in the results of maximal stimulation by pentagastrin varied greatly In three children the maximal output was as much as 75 mM/l and one child was achylic (in two determinations) X ray of the stomach showed soft sign suspect ulcus but no clear ulcer was found Two children had urinary tract infection and one orthostatic proteinuria

Most of the patients had a possible cause for the abdominal pain either in their fundamental character or in their surroundings or in both We have reason to collect about 100 consecutive children with recurrent abdominal pain All the patients will be followed up for several years In this stage of our study it seems that there are many emotional and psychiatric problems among children with recurrent abdominal pain

S Cadranel P Gausset R Platteborse & A Pardou (Brussels) *Relapsing hepatitis with HAA in an infant*

Cases of relapsing hepatitis have been recently described in drug addicts Chronic hepatitis is reported to be more frequent after long incubation (B) than after short incubation (A) hepatitis

We report the case of a female infant who presented with two relapses after an initial onset of fulminant viral hepatitis at the age of 11 months The first attack was treated successfully with multiple exchange transfusion Laboratory data showed evidence of acute necrosis of the liver and serum was strongly positive for HAA A peritoneoscopy and liver biopsy performed 6 weeks after complete recovery from coma did not show any evidence of chronic hepatitis

Disappearance of HAA in the serum occurred after three months but transaminases remained slightly elevated serum immunoglobulins were low Five months later a relapse into acute liver failure with high transaminase levels and positive serum HAA

was again treated with multiple exchange transfusion The child recovered and a second liver biopsy disclosed chronic aggressive hepatitis Following this second hepatitis serum transaminases remained moderately elevated while serum immunoglobulins were low The child developed severe neutropenia with evidence of polyspecific antileucocyte antibody in the blood bone marrow was normal on two occasions Nine months after the initial onset a third attack of acute necrosis of the liver occurred and healed simultaneously

No Ig complement nor Australia AG deposits could be seen on immunofluorescence studies of the liver Thus an autoimmune disease was not proved The possibility of an acquired immunodeficiency remains to be investigated

G C Battaglino V Marcer G Spinosa & V Mengoli (Verona) *A special diet for the treatment of infantile acute diarrhoea*

A double blind treatment study comparing a parmesan cheese ( ) diet (PC) with a PC diet associated with antibiotic therapy per os was performed in 125 infants aged under four months with acute diarrhoea

In the first group of 84 cases (PC diet only) 72 (85 7%) showed normalization of faeces within a period ranging from 1 to 5 days the average lapse being 2 8 days 15 subjects (20 8%) had a relapse when dried cow s milk preparation was introduced into the diet

Enteropathogenic *Escherichia coli* (EPEC) was isolated from the stools of 25 subjects before PC diet and in 13 afterwards

The second group of 41 cases showed recovery for 39 cases (95 2%) *over 1 to 4 days* (mean 1 6) there were 6 relapses (15 3%) EPEC was found in the stools of 14 subjects before therapy and in 7 afterwards In both groups natrium protein urea and glucose in blood and haemoglobin levels controlled before and after therapy showed no significant variations

The AA consider PC diet a good treatment for acute diarrhoea in infants The above mentioned findings however support the effectiveness of antibiotic association during the clinical course without correlation with bacteriological data

( ) *Parmesan cheese composition* (per 100 g of product) proteins 40% as amino acids polypeptides peptones lipids 26% as MCT mainly carbohydrates none minerals 4 5% water 29 5%

C J Rolles & A S McNeish (Birmingham) *Confirming the persistence of gluten sensitivity in young coeliac children by the one hour blood xylose test*

The diagnosis of coeliac disease (CD) now requires the demonstration of continuing gluten sensitivity (1) This is especially necessary when the original diagnosis of CD was made in an infant less than one year old In such a patient the clinical and histological features of CD may be mimicked by secondary disaccharidase deficiency or cow s milk protein intolerance the apparent response to a gluten free diet may represent fortuitous recovery Xylose absorption in coeliac children measured by the one hour blood xylose test (2) may be impaired by gluten reintroduction

Ten coeliac children aged 3–11 months at diagnosis were challenged with oral gluten after 1 month to 4 years on a gluten free diet Repeated blood xylose tests were performed Impaired xylose absorption was demonstrated in nine patients within 1–4 weeks the child who relapsed most slowly had been on a gluten free diet for four years One child diagnosed as coeliac when aged 4 months and on a gluten free diet for 1 year failed to show impaired xylose absorption after 3 months of gluten ingestion Her intestinal biopsy remains normal after a further year of a high gluten diet

a heavy population of immunocytes was observed with a predominance of IgA-containing cells which in most cases outnumbered those producing IgM and IgG However the intestinal IgA cell population was found significantly smaller in infants aged 3–18 months than in children over 18 months

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A T Radzikowski A Malinowski T Zalewski R Aleksandrowicz & A Kukwa (Warsaw) *Cyanosis of hepatic origin Arterial oxygen partial pressure in the course of experimental liver cirrhosis in rabbits*

The main purpose of this paper was to explain the pathophysiology of the liver cirrhosis cyanosis syndrome A motive for these studies was a case of central cyanosis in an eleven year old boy suffering from liver cirrhosis

An experimental model of postnecrotic cirrhosis in rabbits was induced by long term parenteral administration of carbon tetrachloride–rape oil mixture

In seven of nine surviving animals a decrease in arterial p_0 of different degrees was observed In some of these animals hypoxemia was temporary The lung circulatory system was preliminarily examined by injection of plastic masses

On the basis of the results obtained the cause of liver cirrhosis cyanosis syndrome appears to be multiple humorally opened anastomoses between pulmonary arteries and pulmonary veins

M Burdea & I Rusu (Iasi) *Intestinal microflora in the hydromineral metabolism of suckling infants*

Sixty nine suckling infants and four children between one and two years old with acute dehydration (61 with grave dyspepsia and 12 with toxicosis) were investigated for intestinal microflora

The investigated patients were split into four groups a first group of 33 cases with pathogenic coli determined infections a second group represented by five cases with salmonelloses a third of 21 cases with dysentery and a final group of 14 children with Klebsiella staphylococcus and pyocyanic in coprocultures

The gravest signs of dehydration and acidosis were found in the group with salmonelloses followed by the pathogen coli group with dysenteric infection The least serious signs were observed in the fourth group (staphylococcus Klebsiella pyocyanic) The most serious symptoms of salmonellosis etiology diarrhoea are explained through the ability of these germs to invade the intestinal mucosa and to spread throughout the organism through the systemic circulation

Pekka Kuitunen

LETTER TO THE EDITOR

Sir

Henriksson et al (3) report adrenal hypoplasia in a liveborn triploid male infant who weighed 1730 g following a 34 week gestation The occurrence of adrenal hypoplasia in association with intrauterine growth retardation and rare syndromes is of great interest However of greater interest is the occurrence of adrenal hypoplasia with low birth weight whether this low birth weight is associated with multiple congenital anomalies multiple pregnancy prematurity true intrauterine growth retarda tion or a combination of these factors (1 4 6 7) A study of affected infants in three recent reports (1 4 6) reveals 20 infants with a birth weight below 2600 g the sex incidence is 19 males to 6 females

Other reports (2 5 8) have emphasized large placentas with degenerative changes or arborizing amniotic polyps in babies with tri ploidy Henriksson and co-authors state only that the placenta was large Were the gross anomalies described by these other authors (2 5 8) present or not?

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REFERENCES

1 Favara B E Franciosi R A & Miles V Idiopathic adrenal hypoplasia in children *Am J Clin Pathol 57* 87 1972
2 Hashimoto J S Ling S H Millenck J D & Kelly D J Mid trimester pre-eclampsia associated with fetal triploidy report of a case *Am J Obstet Gynecol 116* 88 1973
3 Henriksson P Håkansson L & Sandahl B A liveborn triploid infant *Acta Paediatr Scand 63* 447 1974
4 Laverty C R A Fortune D W & Beischer N A Congenital idiopathic adrenal hypoplasia *Obstet Gynecol 41* 655 1973
5 Schlegel R J Neu R Leao F C Farias E Lewczak P & Gardner L I Arborizing amniotic polyps in triploid conceptuses a diagnostic anatomic lesion? *Am J Obstet Gynecol 96* 357 1966
6 Sperling M A Wolfson A R & Fisher D A Congenital adrenal hypoplasia an isolated defect of organogenesis *J Pediatr 82* 444 1973
7 Szalay G C Adrenal hypoplasia *Am J Clin Pathol 59* 170 1973
8 Walker S Andrews J Gregson N M & Gault W Three further cases of triploidy in man surviving to birth *J Med Genet 10* 135 1973

The Editor has asked dr Henriksson to com ment on the questions raised by dr Szalay

Sir

The placenta in our case showed no gross anomalies Histological examination of the placenta revealed a regular decidua and abundant villi in some areas with syncytial cell buds but no noteworthy chorial cell prolifera tion or signs of mola The umbilical cord con tained three blood vessels and appeared histologically normal

We have discussed the question of adrenal hypoplasia at autopsy of low birth weight in fants particularly those small for gestational age with our pathologists They have not the impression that the adrenals are hypoplastic However they point out the difficulty in ob taining reliable weights of the adrenals Only systematic and carefully standardised dissec tion and weighing of the organs can give com parable data

Per Henriksson

NEW BOOKS RECEIVED

J L Melnick (ed) *Progress in medical virology Monographs in virology* Vol 16 363 pp illus S Karger AB Basel 1973 sFr 98 –

E R Stiehm & V A Fulginiti (eds) *Immunologic disorders in infants and children* 637 pp illus W B Saunders Company Ltd London 1973 £11 05

I Kolvin R C Mac Keith & S R Meadow (eds) *Bladder control and enuresis* Clinics in Developmental Medicine Nos 48/49 328 pp illus Spastics International Medical Publications William Heinemann Medical Books Ltd London 1973 £6 20

R S Illingworth *Basic developmental screening 0–2 years* 58 pp illus Blackwell Scientific Publications Oxford 1973 £0 80

K H Marshall & A A Fanaroff *Care of the high risk neonate* 358 pp illus W B Saunders Company Ltd London 1973 £6 40

R B Taylor *A primer of clinical symptoms* 238 pp illus Harper & Row Publishers Inc Hagerstown 1973 $10 95

G Erdmann *Erkaltungskrankheiten im Kindesalter Infekte der Luftwege* 103 pp illus Gustav Fischer Verlag Stuttgart 1973 DM 8 –

M E Avery & B D Fletcher *The lung and its dis orders in the newborn infant* 3rd ed 361 pp illus Vol 1 in the Series Major Problems in Clinical Pedia trics (A J Schaffer consulting ed) W B Saunders Company Ltd Philadelphia London and Toronto 1974 £5 35

Wallace W McCrory *Developmental nephrology* Harvard University Press Cambridge Mass 1973 $12 –

Melvin M Grumbach G D Grave & F E Mayer *The control of the onset of puberty* 484 pp illus John Wiley & Sons Ltd Chichester 1974 £12 00

Maturation of fetal body systems World Health Organization Technical Report Series No 540 33 pp Report of a WHO Scientific Group Geneva 1974

BOOK REVIEWS

L M Solomon & N B Esterly *Neonatal dermatology* Vol IX in the series Major Problems in Clinical Pediatrics 714 pp illus W B Saunders Company Philadelphia London and Toronto 1973 £7 05

This monograph dealing with skin disorders in the newborn is part of a series on different aspects of clinical paediatrics The authors have expanded the subject from earlier review articles and compiled it in book form

The first chapters deal with structure and function of the skin in the neonate Then follows a description of elementary skin lesions and a short but good survey of diagnostic procedures The section on general therapeutic principles gives good guidelines and helps to avoid some of the most often encountered pitfalls The authors advocate a sound restrictiveness in using powerful medications such as fluorinated corticoids in infants Also topically applied antibiotics might have systemic effects when the skin is eroded

Thirteen chapters dedicated to the various dermatologic conditions in the neonate follow after this introductory part Like other textbooks on dermatology classification of the disorders has not been made on an etiological basis This is unfortunate but it is an almost impossible task and the authors have instead chosen to group the diseases according to the most frequent and obvious present signs This might be a principle as good as any but it leads to some inconsequences and confusion and you often have to use the index to know where to find a certain disorder

Thus for instance the vascular nevus has been withdrawn from the chapter on nevi and tumours and placed with erythemas of unknown origin and vasculitis The melanocytic nevi can be found in the chapter on pigmentary disorders and incontinentia pigmenti has been grouped with the vesico bullous eruptions On the other hand some diseases such as for instance toxic epidermal necrolysis are treated in more than one chapter The genetic dermatoses are spread over most of the chapters This of course is a consequence of the authors principle of selection but it makes it difficult to deal with problems common to all genetic disorders such as genetic family counseling This aspect is briefly mentioned in some of the hereditary disorders but not for instance in the section on epidermolysis bullosa

In the chapter on eczema the important fact that atopic dermatitis is very rare during the first month is stressed and strong criteria are required to make the diagnosis in this time period An interesting suggestion is that seborrhoeic dermatitis in the neonate could be a very early stage of atopic dermatitis in some cases

The section on scaling dermatoses mainly discusses the different forms of ichthyosis and their treatment The ichthyosis forms seen in the newborn all belong to the congenital group and they are relatively rare The differential diagnosis may be difficult and a skin biopsy helpful The therapy is not as successful as in the vulgaris type and topical treatment with urea preparations or lubricants improves the scaling tendency but does not influence the erythrodermia In very severe cases steroids and cytostatics may be necessary

The chapter on vesico-bullous eruptions contains a mixture of non related disorders ranging from infections (where because of unfortunate typography candida and arthropod induced blisters have been grouped under the heading Epidermal viral lesions) to epidermolysis bullosa and acrodermatitis enteropathica Epidermolysis bullosa is a group of diseases where a generally accepted system of classification has not yet been achieved A panel discussion was held at the first meeting of the European Society of Pediatric Dermatology in London in July 1974 to recommend nomenclature and classification The authors use the classification of Pearson in Dermatology in *General Medicine* edited by Fitzpatric (1971) and separate the non-scarring types from the scarring ones A Scandinavian reader will perhaps miss a reference to the work of Gedde Dahl (1971) concerning the complex and fascinating genetic patterns of these diseases

The recently reported side effects of iodoquine therapy in acrodermatitis enteropathica causing retinopathy and visual impairment in several cases is not mentioned by the authors

The book gives data on a great number or rare syndromes which the dermatologist seldom meets The authors have intended to be rather comprehensive and they seem to have been successful in this respect A few disorders however have been omitted Morbus Fabry (glycolipid lipidosis) and the Gianotti Crosti syndrome are not mentioned and pityriasis rosea although mainly a desease of young adults can occur in infancy

The references given after each chapter are very adequate and up-to-date The illustrations are of somewhat differing quality There are several good pictures but many of them do not fulfill normal standards of a text book of dermatology This is also true for the colour pictures

Summing up this is a useful monograph for both the paediatrician and the dermatologist and it certainly has a place in the reference library of the clinic

Bo Ljunggren

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The Editorial Board of Acta Paediatrica Scandinavica wishes to express its sincere gratitude to the following persons outside the Advisory Board who have acted as referees during the past year. The standard of the journal depends to a very large extent on the skill and interest of these reviewers.

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Acta Pædiatr Scand 64 161–171 1975

A TWO CENTURY PERSPECTIVE OF SOME MAJOR NUTRITIONAL DEFICIENCY DISEASES IN CHILDHOOD

BO VAHLQUIST

From the Department of Paediatrics University Hospital Uppsala Sweden

ABSTRACT Vahlquist B (Department of Paediatrics University Hospital Uppsala Sweden) A two-century perspective of some major nutritional deficiency diseases in childhood Acta Paediatr Scand 64 161 1975.—In this review dealing with historical aspects and the present day situation in developing countries three major nutritional deficiencies among children are discussed namely rickets iron deficiency anaemia and protein energy malnutrition (PEM)

KEY WORDS Developing countries rickets iron deficiency anaemia protein energy malnutrition (PEM)

The contents of Rosen von Rosenstein s famous Textbook on Pediatrics (The Diseases of Children and their Remedies) first published in 1764 reflect the pediatric problems of the time (25 29) It is no wonder that the majority of illnesses listed fall into the domain of communicable diseases Their often dramatic course and their contagiousness often manifesting itself in peaks of recurring epidemics attracted the special attention of physicians and laymen alike Let us not forget however that much suffering and a vast number of lost lives at that time had their origin in malnutrition and that this was particularly true for the children In many ways the situation in 18th century Sweden was the same as we see it in developing countries today

I shall limit my presentation to three major nutritional deficiencies of particular importance in childhood a vitamin deficiency—rickets a mineral deficiency—iron deficiency anaemia and a more profound nutritional deficiency namely protein-energy malnutrition

Lecture given at a meeting of the Swedish Pediatric Society commemorating Rosén von Rosenstein May 11 1974

RICKETS

First a brief summary of our knowledge concerning the etiology clinical picture and biochemistry and prevention as it stands in 1974

Etiology

Deficiency of *vitamin D in* the body of the fast growing young child Many traditional diets have an unsatisfactory or even a very low content of vitamin D With rare exceptions however they contain precursors to the vitamin which are converted to vitamin D in the body when the *naked skin* is exposed to the sun Thus the prerequisite for rickets is the *unfortunate combination* of a diet very low in vitamin D and non-exposure of *the naked skin* to the sun

Clinical picture

This is well known to everyone I shall *limit myself* here to presenting a schematic illustration (Fig 1)

Biochemistry

Time does not permit more than the presentation of a schematic diagram (Fig 2) *Incidentally* it is worthy of mention that recent discoveries clarifying the *metabolism* of vitamin D have led to a discussion as to whether the

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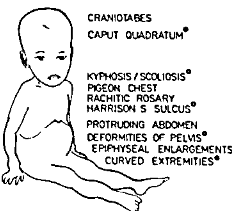


Fig 1 Rickets—schematic drawing of clinical features

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Vitamin D is a very potent antirachitic agent Daily administration of 400 IU of the vitamin i e 1/100 of a milligram is enough to keep rickets away even in the most sensitive age group children below 2–3 years And if the disease has already become manifest it can be cured by a 10 fold increase of this dosage over a few weeks or by administration of one single massive dose of 10 mg of vitamin D

Historical aspects

As in so many other conditions rickets seems to have existed for as long as bodily remains and naive reproduction permit an evaluation The classical description stems from the British scholar Frances Glisson a pupil of Harvey in the year 1650 (Fig 3) His treatise—in Latin—is said to depict the clinical

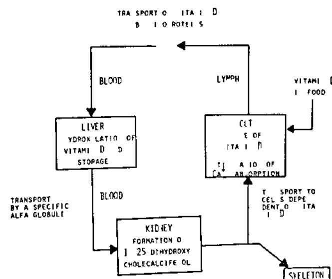


Fig 2 Vitamin D—uptake transport and conversion (18)



Fig 3 Cover illustration of the classical work on rickets by Glisson (12)

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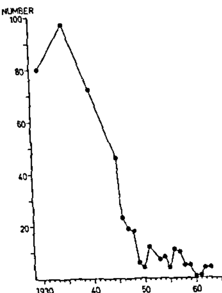


Fig 4 Decline in prevalence of rickets in Sweden as witnessed at a paediatric clinic (15)

satisfactory and swaddling of children was widely practised rickets was omnipresent in large parts of Europe Later on industrialization and rapid urbanization with the development of highly unhealthy and widespread slums continued to form a fertile ground for the disease

At this time however a new era dawned Cod liver oil as a remedy for rickets was first mentioned in the early 19th century (27) and then steadily gained ground in European countries during the 19th and the early part of the 20th century Decisive progress then followed about 50 years ago In 1919 Mellanby of the UK first reported on his studies of experimental rickets in dogs which he found could be prevented and cured by certain fats notably cod liver oil (21) Three years later McCollum of the US one of the pioneers in nutrition research provided evidence that it was not vitamin A but another fat soluble factor designated vitamin D which is essential for the normal ossification of the skeleton (20) In 1932 finally the German scientist Windaus clarified the chemical structure of what is now known as vitamin D_2 Thus at a time when Europe was still suffering from the aftermaths

Table 1 *Prevalence of rickets in two African countries (15)*

Age (months)	Ethiopia (% n = 99)	Tunisia (% n = 306)
0–5	19	26
6–11	36	37
12–23	10	23
24–35	15	5
36–47	4	0

of World War I a major breakthrough was achieved in the fight against a disease which has scourged children over millenniums

In Sweden today rickets is to all intents and purposes completely eradicated Still around 1935 about 20% of all children 0–2 years of age admitted to the Paediatric Clinic of Uppsala showed clinical signs of rickets But then a dramatic change for the better took place in the late 1930 s and the 1940 s that is at the time when concentrated effective preparations of vitamin D became easily available and when the child health clinic organization gained strength and momentum (Fig 4)

Developing countries

Two hundred years ago Sweden was a pre industrial society What is the rickets situation in pre industrial societies of our time? For comparison I would like to present the situation in two African countries (Table 1) Thus it is a paradox that rickets which was once known as a disease of the temperate regions of the world is today mainly a disease of the subtropics and the tropics

IRON DEFICIENCY ANAEMIA

Again I will first give a brief summary of our present knowledge concerning the etiology clinical picture biochemistry and prevention of this disease

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The cause of the disease is lack of iron due to a low iron uptake high iron losses and/or rapid growth

A low iron uptake may be due to a low content of iron in the diet and/or low availability of the iron contained in the

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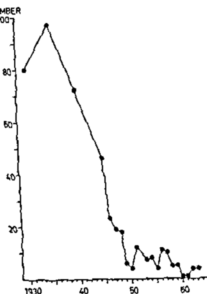


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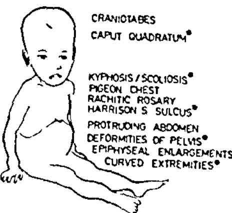


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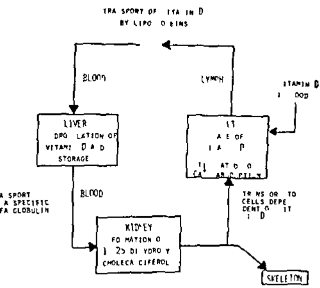


Fig. 2 Vitamin D—uptake transport and conversion (18)



Fig 7 Blood letting in medieval times (11)

dyspnea etc are nonspecific in nature Only with the advent of laboratory methods did it become possible to make an accurate diagnosis and to gauge the prevalence and the severity of anaemias Reasonably reliable methods for erythrocyte counting (Vierordt modified by Malassez 1874) and for hemoglobin determination (Gower 1875) were introduced only in the 1870 s and it took some time before they gained wide application For the period up to that time we are left very much in the dark with respect to the role of anaemia as a public health problem

There are good reasons however to believe that anaemias particularly the iron deficiency form have been widespread The medical profession may unwittingly have contributed to this state of affairs Alongside emetics and laxatives blood letting was a standard therapeutic procedure from times long past up to the mid 19th century (Fig 7) Indeed the custom of blood letting concluded over half a century—during and after the Napoleonic period—in an almost cataclysmic way It is remarkable that the effect of large and frequently repeated phlebotomies often complemented by systematic cupping and application of leeches as a cause of long standing severe iron deficiency anaemia in the survivors has hardly been commented on at all in the literature

In his brilliant essays on the history of medicine the late Professor Robin Fåhræus of this University has given a vivid description of this kind of therapy intended to rid the blood of flogistic materia and to reduce tension in sthenic bodies (11) The famous French 19th century physician Bouillaud considered that the treatment of pneumonia should include phlebotomies morning and afternoon during the first two days and then once daily And it was not a symbolic rite but should be a saignée a blanc —bleeding almost to fainting It could well be that during one week the body was deprived of blood to an amount of several litres And children were not exempt A 7 year-old child of the nobility with pleurisy underwent phlebotomy 13 times and was then said after two weeks to be in good health The amazing thing was that phlebotomies were also used in situations which today seem completely absurd e g before surgery and delivery and as a remedy for internal bleeding etc

The wealthy townspeople were perhaps exposed to a greater risk of overzealous phlebotomy than the rest of the society However cupping at least was certainly widespread in rural areas also In Sweden Dr Abraham Back President of the Collegium Medicum during the latter part of the 18th century advocated that the parish clerks be instructed in cupping in order to have at least one person in each parish with knowledge of this procedure

Added to this was the widespread treatment with blood sucking leeches Let me quote just one figure from France In the year 1833 in that country alone 41 million leeches were imported for medical use mostly from Eastern Europe but also from other countries The

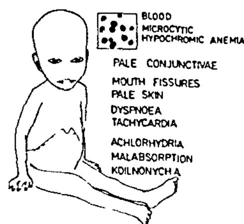


Fig 5 Iron deficiency anaemia—schematic drawing of clinical features

food The healthy fullterm newborn baby starts life with a fairly large iron depot which is found mainly in the circulating red cell mass This depot covers all the needs for growth and metabolism during the first three months but if after this period the iron of the food is low over several months e g by unsupplemented milk feeding then anaemia may ensue

Abnormal iron losses are always synonymous with abnormal blood losses In children in our part of the world such a situation is not a common one In the developing countries blood losses from the gut due to hook worm infestation is in some areas a major contributing factor to severe iron deficiency

The child is especially vulnerable to iron deficiency owing to its rapid growth Prematurely born or light for date babies run a special risk of developing anaemia Women in fertile age are also vulnerable owing to menstrual blood loss and reproduction Adult males are the fortunate ones from the iron point of view

A special word should be said about the availability of the iron in the food This varies a lot from one foodstuff to another However in an ordinary mixed diet a kind of balance is rapidly reached in the stomach with the formation of two pools one of heme iron (blood meat etc) and one of non heme iron (bread legumes milk etc) (14)

The availability of iron also depends on the gross composition of the food The more animal foodstuffs the better the iron absorption WHO now recommends a daily intake for children 1–12 years of only 5 mg iron per day if animal food covers more than 25% of the calories whereas this figure is doubled (10 mg) if animal food covers less than 10% of the calories (22) For the developing countries the latter figure is certainly the one that is widely applicable

Clinical picture

I shall limit myself to a schematic drawing (Fig 5)

Biochemistry

Again time does not permit more than scratching on the *surface*

At virtually every known step of iron metabolism including storage transport biosynthesis and degradation the ferrous to ferric cycles play a dominant role in the multimetabolic pathways of the most important metal constituent in all living systems This is a quotation from a paper by Frieden which gives a condensed and up to date review (10) I should also like to show a diagram from another article (19) (Fig 6)

Prevention and cure

The sole and adequate remedy for iron deficiency anaemia is—iron Obviously any special causative factors such as abnormal blood losses should at the same time be brought under control

On a gross average the utilization of iron from commonly used medicinal iron preparations is about 5% This increases considerably up to 15–20% in states of iron deficiency On a gross average the amount of iron needed to cure anaemia in a 2 year-old child is in the order of 50 mg per day distributed in several doses and continued during 4–6 weeks

Historical aspects

And now again a look backwards In the medical literature from ancient times up to the 19th century sporadic articles and monographs dealing with chlorosis and other blood dyscrasias can be found One example is the contribution by Francis Hoffman in 1731 Dissertatio de Genuina Chlorosis *Indole* Origine et Curatione

On the whole however the anaemias occupy a rather humble place in the history of medicine particularly as far as children are concerned In Rosen von Rosenstein s *chef d œuvre* which in many ways is so exhaustive not a single paragraph is devoted to anaemias

In all probability the explanation of such a neglect is the fact that signs such as paleness

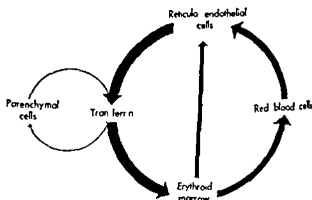


Fig 6 Iron metabolism—schematic presentation
Source S R Lynch et al *Iron in Biochemistry and Medicine* Academic Press London and New York 1974 (19)

Table 2 *PEM definitions (5)*

Category	Body weight as % of standard (W/A)
Mild PEM	<90 >80
Moderate PEM	<80 >60
Nutritional marasmus	<60 no oedema
Kwashiorkor	<80 >60 oedema
Marasmic kwashiorkor	<60 oedema

Note Low W/A in PEM can be explained by 2 factors low W/H (leanness) and low H/A (short stature) (W/A= W/H×H/A) If following nutrition rehabilitation W/H returns to normal but H/A remains low this means nutritional dwarfing

picture if severe is then called marasmus But it may also strike protein much more than calories if so the disease picture named kwashiorkor may develop

For reasons of simplicity the definition of various degrees and types of PEM is worked out on the basis of weight for age and presence or absence of oedema (Table 2)

Clinical picture

The clinical symptomatology of PEM is described in two schematic illustrations taken from a monograph by Professor Jelliffe (Fig 9)

It should be noted that in addition to the clinical signs given in this picture there is often an array of other signs expressing various types of deficiencies—vitamins minerals at times also essential fatty acids I shall only mention one of these in this context the vitamin A deficiency In those areas of the world where the prevalence of severe PEM and particularly of kwashiorkor is high and the dietary intake of vitamin A is low e g in the Middle East and in South East Asia the occurrence of blindness in survivors of a combination of PEM and severe vitamin A deficiency is quite common

Biochemistry and function

Some features related to the composition of the blood plasma in protein-energy malnutrition are collected in the following table (Table 3)

As far as function is concerned in severe PEM even the fundamental ones are affected The basic metabolic rate is decreased and the need of oxygen carrying hemoglobin is reduced The anaemia of PEM *per se* is often aggravated through coexisting deficiencies of iron folate B_{12} and/or trace metals

The gut in severe PEM is characterized by atrophy of the mucosa and impaired absorption of a number of nutrients and their metabolites (disacchandes lipids vitamin B_{12} iron etc) (34) Even the nervous system may suffer In kwashiorkor gross changes in biochemistry and function have been noted during the acute stage (6 7)

Of special interest is the relationship between PEM and infections/parasitoses In the developing countries all kinds of infections are rampant and go to massive attack on the children as soon as breast feeding ceases Under these circumstances there is almost always a vicious circle between marasmus and an almost endless array of diarrhoeas and other types of infections In the same way kwashiorkor is very often preceded by infections such as diarrheic conditions and measles

Only recently has systematic research begun to shed light on the mechanisms involved in nutritional immuno-deficiency (Table 4) (8 24 33)

Prevalence

Industrial societies

There are pockets of glaring poverty even in many industrial countries but they seldom reach such a degree that because of poverty alone the basic protein-energy needs cannot be covered And in countries like the Scandinavian ones a malnourished young child—a rare

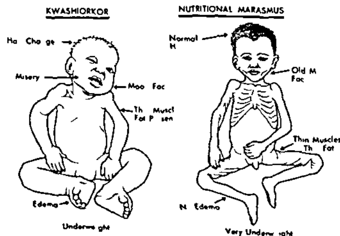


Fig 9 Protein energy malnutrition (PEM) —schematic drawing of clinical features
Source D B Jelliffe *Child nutrition in Developing Countries* U S Government Printing Office Washington 1968 (16)

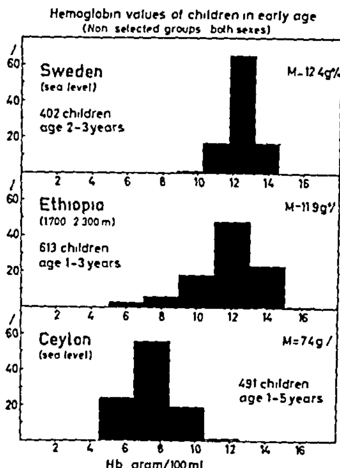


Fig 8 Prevalence of iron deficiency anaemia in pre school children in three countries (30)

Swedish leeches were said to be particularly effective!

Iron deficiency anaemia is a preventable disease just as is rickets There is good reason to state that the prevalence of iron deficiency anaemia just as the prevalence of rickets can be used as an index of the efficiency of the health services in a given country When I carried out studies leading to my thesis some 35 years ago (28) iron deficiency anaemia was still quite common among children particularly children in the first few years of life Today it is a rarity This was demonstrated in the study from the province of Vasterbotten by Samuelsson & Sjolin in 1972 (26) In a random sample of 1481 children comprising the age groups 1 4 8 and 13 years only three i e 0 2%, exhibited overt iron deficiency anaemia Several factors have contributed to this favourable state of affairs including systematic iron prophylaxis for children underweight at birth iron fortification of infant formulae regular introduction of semi solid foods at 3 months of age and an improved and more varied food pattern favouring a higher iron intake

Developing countries

As with rickets let us now briefly scrutinize the situation of anaemia in the pre industrial societies of the world The following figure may serve to illustrate the situation (Fig 8) (30)

The high prevalence of anaemia among pre schoolers in Ceylon (nowadays Sri Lanka) is characteristic of a great many of the developing countries whereas Ethiopia in this respect forms an exception because of the high iron content of the food

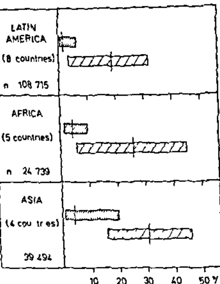
Iron deficiency anaemia lessens the resistance to disease and reduces the working capacity What can be done to eradicate it in those regions of the world which are most afflicted that is the developing countries? A daily intake of iron preparations or of iron enriched western type foods is hardly ever practicable except in a small privileged group Efforts are made though still on a limited scale to enrich indispensable centrally produced consumer goods with iron e g salt sugar fish sauce etc (22) All measures serving the eradication of hook worm infestation and further health education promoting better use of the meagre resources to produce nutritious food act in the same direction It is hoped that one day the factor or factors responsible for the enhancing effect of animal food on iron absorption will be defined and available for practical use

PROTEIN ENERGY MALNUTRITION (PEM)

A recent Glossary in Nutrition presented by WHO (9) gives the following definition

A range of pathological conditions arising from coincidental lack in varying proportions of protein and calories occurring most frequently in infants and young children and commonly associated with infections

The nutritional deficiency in PEM can strike protein and calories in a balanced way and the ensuing disease



Po t pre ole ce of PEM community surveys 1963 72 (≯1000 ch ld e /study)

Se ere forms range and med an

moderate

Fig 10 Pre alence of moderate and severe PEM Presen tat on based on a number of different field studies Diagram ba ed on a table by Bengoa ( )

of institutionalized young children in those days is given by Girtanner in his textbook from 1794 (13) When discussing the dangers of arti ficial feeding at an early age he writes

Zu Rouen wurde der Versuch mit der allergrossten Vor cht angestellt Das Haus war ausser der Stadt mit Baumen umgeben und die Kinder hatten die sorgfaltig te Wartung Man suchte dieselben durch Kuhmilch und Mehlbrey gross zu ziehen Aber der Versuch misslang Von hundert und zwey und dreyssig Kindern waren nach anderthalb Jahren nur noch dreyzehen am Leben und auch diese blieben schwachlich und starben bald nach her

However as always when we come to facts and figures related to the situation at large we are very much left in the darkness Sweden introduced vital health statistics as early as in 1749 earlier than any other country of the world But registration of causes of death be came compulsory only in 1911 and certainly also long after this time the correctness of diagnosis and classification especially for var ious types of malnutrition left much to be desired It has only been since the end of World War II that the pediatric textbooks have gradually abandoned a time honoured but dis parate and often confu ing terminology includ ing words such as atrophy atrepsia and ca chexia or spectacular names in vernacular language such as *Milchnahrschaden* and *Mehlnahrschaden* The concept of protein energy malnutrition with its different sub groupings marasmus and kwashiorkor being the most severe forms may at times represent an oversimplification However on the whole this new concept has done much to clarify matters and make possible a standard classifi cation

PEM causes a multitude of ill effects which

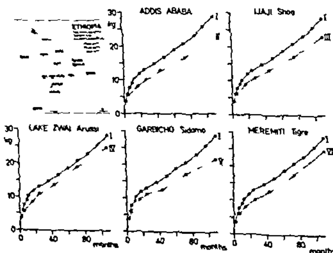


Fig 11 Weight increase in children from five different parts of Ethiopia (II–VI) as compared with that in Swedish children (I) (1)

Table 3 *Blood plasma biochemistry in severe PEM (32)*

	Marasmus	Kwashiorkor
Total protein	Normal/slightly decreased	Low
Albumin	Normal/slightly decreased	Low
Retinol binding protein (RBP)	Normal	Low
Transferrin	Slightly decreased	Low
Ess amino acids	Normal	Low
Lipids (lipoprotein cholesterol triglycerides)	Normal	Low
Enzymes	Normal/slightly decreased	Low
Glucose	Normal/slightly decreased	Low

event in itself—will immediately arouse suspicion of an underlying disease

Pre industrial societies

There are some 500 million children below 5 years of age in the world today Of these 80% live in the developing countries and for them PEM is more the rule than the exception The prevalence figures vary considerably from one region to another (Fig 10) A rough estimate (2) gives the following figure on a world basis Severe PEM 9 4 million moderate PEM 89 million, together almost 100 million or about 25% of all children below 5 years in developing countries Among the remainder comparatively few are completely free from signs of PEM—at least this is true for most of the African countries To corroborate this statement I shall present some data on the physical development of Ethiopian children as they appear in non famine periods i e in periods of so to speak traditional moderate hunger (Fig 11)

Table 4 *Mechanisms involved in nutritional immuno deficiency (32)*

HUMORAL IMMUNITY

Immunoglobulin fractions (IgG IgA IgD IgE) generally well increased during infection

Certain other specific protein fractions (Complement C_3 transferrin RBP) reduced

Antibody forming capacity in relation to *certain* antigen (e g yellow fever S typhi) reduced

CELLULAR IMMUNITY

Thymus atrophic histologically changed

Tuberculin positivity greatly reduced

DNCB sensitivity greatly reduced

PHA stimulation reduced blastic transformation of lymphocytes

Phagocytes reduced

Clinical observations serious course of certain bacterial (e g TB) and viral (e g measles) infections

Sequelae

PEM is one of the leading causes of death in pre school ages but it does not stop there In the survivors PEM may cause life long sequelae We have already discussed the impairment of physical development in general Of particular importance is what happens to the nervous system and to the mental functions In a recent thesis by Dr Gunnar Engsner based on experience from the SIDA supported Ethiopian Nutrition Institute the effects of severe protein-energy malnutrition on brain growth have been studied with modern technique Especially in kwashiorkor the situation in this respect in the acute stage of the disease shows a striking deviation from the normal (6)

In the culture of poverty there is usually a combination of malnutrition infection and understimulation It is well documented that these factors in combination exert lasting unfavourable influence on the mental and behavioural development of the children concerned It is not so clear to what extent malnutrition alone may have such an effect This problem was discussed at some length at a recent symposium in Saltsjobaden Sweden (4)

Historical Aspects

And now what has been the history of PEM in Europe over the centuries? For reasons already touched upon we can assume that two centuries ago PEM often in combination with diarrhoeas and other infections was one of the great killers of young children in our part of the world also A pathetic reminder of the fate

REFERENCES

1 Belew M Jacobsson K Tornell G Uppsall L Zaar B & Vahlquist B Anthropometric clinical and biochemical studies in children from five different regions of Ethiopia *J Trop Pediatr Env Child Health 18* 746 197?

2 Bengoa J M The problem of malnutrition *WHO Chron 28* 3 1974

3 Cameron M & Hofvander Y *Manual on feeding infants and young children* PAG Document 1 14/26 1971

4 Cravioto J Hambræus L & Vahlquist B (eds) *Early malnutrition and mental development* Symposia of the Swedish Nutrition Foundation XII Almqvist & Wiksell Uppsala 1974

5 Editorial Classification of infantile malnutrition Lancet *II* 30 1970

6 Engsner G Habte D Sjögren I & Vahlquist B Brain growth in children with kwashiorkor A study using head circumference measurements transillumination and ultrasonic echo ventriculography *Acta Paediatr Scand 63* 687 1974

7 Engsner G & Woldemariam T Motor nerve conduction velocity in marasmus and in kwashiorkor *Neuropadiatrie 5* 34 1974

8 Faulk W P Nutrition and immunity *Nature 250* 283 1974

9 *Food and Nutrition Terminology* Definitions of selected terms and expressions in current use WHO/FAO with the collaboration of the IUNS NUTR /73 1973

10 Frieden E The ferrous to ferric cycles in iron metabolism *Nutr Rev 31* 41 1973

11 Fåhræus R *Lakekonstens histo ia* del III Albert Bonniers Forlag Stockholm 1950

12 Glisson F *De Rachitide si e Morbo Puerili qui vul go The Rickets dictu* Tractatus London 1650

13 Girtanner Ch *Abhandlung uber die Krankheiten der Kinde und uber die physische Erziehung derselben* Berlin 1794

14 Hallberg L & Bjorn Rasmussen E Determination of iron absorption from whole diet A new two-pool model using two radioiron isotopes given as haem and non haem iron *Scand J Haematol 9* 193 197?

15 Hof ander Y & Vahlquist B Rakit i soliga lander *Na i gsf rskn ng 13* 48 1969

16 Jelliffe D B *Child nu trition in de eloping count ies* 1st ed U S Government Printing Office Washington 1968

17 Jelliffe D B & Jelliffe E F P *Nutrit on program me f p es hool child en* Reports of a conference held in Zagreb Yugoslavia August 3–6 1971 Institute of Public Health of Croatia Zagreb 1973

18 Karlsson F A & Peterson P A Vitamin D—några a pekter på dess verkningsmekanism *Naringsforskning 15* 66 1971

19 Lynch S R Lipschitz D A Bothwell T H & Charlton R W Iron and the reticulo-endothelial system *In* A Jacobs & M Worwood (eds) *Iron in b hemist y and med cine* Academ c Press London and New York 1974 p 563

20 McCollum E V Simmonds N Becker J E Shipley P G & Park E A J Biol Chem 54 249 1972 quoted from E V McCollum *A History of Nutrition* The Johns Hopkins University The Riverside Press Cambridge 1957

21 Mellanby E Lancet (London) i 407 1919 quoted from E V McCollum *A History of Nutrition* The Johns Hopkins University The Riverside Press Cambridge 1957

22 *Nutritional Anaemias* Report of a WHO Group of Experts *WHO Tech Rep Ser No 503* 1972

23 Review Calcium transport and mobilization mediated by 1 25-dihydroxycholecalciferol *Nutr Rev 31* 58 1973

24 Review Cell mediated immunity and resistance to infection *WHO Chron 27* 531 1973

25 Rosén N *Underrattelser om Barn Sjukdomar och deras Bote Medel Tilforene styckevis utgifne uti de små Almanachorna nu samlade tilokte och forbattrade* Lars Salvius Stockholm 1764

26 Samuelsson G & Sjolin S An epidemiological study of child health and nutrition in a northern Swedish county IV Haematological investigations especially in regard to iron deficiency anaemia *Acta Paediatr Scand 61* 63 197?

27 Scheutte D Arch med Erfahrung 79 80 1874 quoted from E V McCollum *A History of Nutrition* The Johns Hopkins University The Riverside Press Cambridge 1957

28 Vahlquist B Das Serumeisen—eine padiatrisch klinische und experimentelle Studie Dissertation *Acta Paediatr Scand 28* Suppl 5 1941

29 Vahlquist B & Wallgren A (eds) *Nils Rosen von Rosenstein and his Textbook on Paediatrics* Almqvist & Wiksell Uppsala 1964

30 Vahlquist B Occurrence of nutritional anaemias in children *In* G Blix (ed) *Occurrence Causes and Prevention of Nutritional Anaemias* Symposia of the Swedish Nutrition Foundation VI Almqvist & Wiksell Uppsala 1968 p 66

31 Vahlquist B (ed) *Nutrition—A priority in African development* The Dag Hammarskjold Foundation Almqvist & Wiksell Uppsala 197?

32 Vahlquist B Hungerdystrophie (Protein Energy Malnutrition PEM) unter besonderer Berucksichtigung der Ernahrungssituation in den Entwicklungslandern Monatsschr Kinderheilkd (in press)

33 Vitale J J & Good R A Nutrition and Immunology—Introduction (Symposium) *Am J Clin Nut 27* 623 1974

34 Viteri F E Flores J M Alvarado J & Behar M Intestinal malabsorption in malnourished children before and during recovery *Am J Dig Dis 18* 201 1973

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not only mean suffering for children and their families alike, but also act as a real obstacle to social and economic progress in the pre industrial societies This is the situation in ordinary times The problem rises to almost overwhelming proportions when the normal chronic malnutrition goes out of control through famine disasters, as we are witnessing at the present time in large areas of Africa and Asia

Prevention

All the diseases I have dealt with PEM included are clearly preventable and therefore in a sense completely intolerable in a world which knows all that is needed to know in this respect and which has stated through the UN Declaration of Human Rights that everyone has the right to sufficient and proper food

And yet the opinions with respect to appropriate practical measures differ widely Many claim that PEM is a consequence of poverty and can be eradicated only through raising living standards up to a much higher level than is the fate for innumerable millions of fellow world citizens today No objection can be raised against such an attitude and every effort to eradicate social and economic injustices for the benefit of health and nutritional wellbeing as well as for other reasons is warranted That a radical change in political structure can create a completely new situation in this respect has been amply proven in China and also in Cuba

However for many countries it will take a long time to arrive at a solution along these lines alone In the meantime other approaches implying short cuts for the benefit of vulnerable groups will have to be used extensively The role of health personnel is of great importance in this respect In order to fulfil their tasks in the most efficient way these personnel must have a broad and thorough knowledge of nutrition and also of the most efficient methods for communicating this knowledge It is much easier to give shots of penicillin and prescribe treatment for diarrhoea than to in struct mothers in a realistic way of the use o local staples for preparing nutritious weanin foods or administering a food program fo pre school children And yet so far these latte activities if undertaken at all have often bee assigned to the most junior least trained staf It is high time that a change took place—nutri tion should become a major issue in all kind of training including that of health personnel I shall not go into details of practical program here For those interested there is ample in formation to be found in the literature i monographs by Jelliffe and Jelliffe (17) Came ron & Hofvander (3) and others and also t the Proceedings published in 1972 from th Dag Hammarskjold Symposium Nutrition a a Priority in African Development (31)

I have come to the end of my presentation Two centuries is just a flash in the history o mankind Yet the last two centuries have seen a revolution much more far reaching than un der any earlier period Rosen von Rosenstein was a portal figure as far as pediatrics is con cerned but as we see it today he had very few really effective and reliable cures to recom mend

When my father studied medicine here in Uppsala in the 1880 s the really active and efficient remedies available to the physician had increased a little but could still be counted on the fingers of one hand—smallpox vaccina tion and drugs such as digitalis quinine chloral and salicylic acid

Even at the turn of the century as the say ing goes the chance of becoming better or worse after consultation with a doctor was still in the order of fifty fifty And *now*—the av alanche of new efficient methods for diagnosis cure and prevention! It has been my great privilege to have as teachers and friends some of the great pioneers in the advancement of medicine To them I dedicate this lecture and I do this with three particular names in mind Arvid Wallgren Gunnar Blix and Jan Waldenstrom

Table 1 *Composition of formulas*

	Formula 5018D	Formula 5018E
Concentration (kcal/100 ml)	54	100
Major constituents (g/100 ml)		
Protein	1 53	1 50
Fat	2 89	5 58
Carbohydrate	5 50	11 70
Mineral content per liter		
Calcium (mg)	610	610
Phosphorus (mg)	450	490
Sodium (meq)	14	14
Potassium (meq)	5	6
Chloride (meq)	16	16
Magnesium (mg)	70	79
Iron (mg)	14 5	14 0
Vitamin content per liter		
Vitamin A		
Thiamin (µg)	860	860
Riboflavin (µg)	1 350	_ 300
Niacin (µg)	5 040	5 300
Pyridoxine (µg)	400	430
Ascorbic acid (mg)	50	61
Vitamin D		

Because vitamins A and D were not included a daily supplement was given throughout the study This supplement (Tri Vi Flor[2]) provided in 1 0 ml 2 000 I U vitamin A 400 I U vitamin D 60 mg vitamin C and 0 5 mg fluoride (from sodium fluoride)

caloric concentration—one 67 and the other 133 kcal/100 ml Parents of these infants were given no instructions regarding desirable volumes of formula intake for their infants In the interval 8 through 41 days of age caloric intake and rate of growth (i e gain in weight and gain in length) were greater by infants receiving the more concentrated feeding After 41 days of age both caloric intake and rate of growth were similar with the two feedings

The present study was undertaken to determine caloric intake and rate of growth of normal infants fed formulas of similar composition except that caloric concentration was 54 or 100 kcal/100 ml These formulas differ less markedly from conventional 67 kcal/100 ml formulas than was true of the previously studied highly concentrated formula supplying 133 kcal/100 ml

On the basis of observations recorded here and several assumptions we have speculated about the chemical composition of the weight gained by infants in the two feeding groups (see Discussion)

SUBJECTS

Normal fullterm female Caucasian infants with birth weights greater than 2 500 g were enrolled in the study during the first 9 days after birth The majority of the subjects were daughters of students or younger faculty of the University of Iowa

Between July 28 1971 and June 30 1972 all females available for enrollment in a study were assigned alternately to feeding groups 5018D and 5018E

FEEDINGS PROCEDURES AND METHODS

Formulas

During the first few days after birth nearly all of the infants were fed Similac or Enfamil[2] and several of the infants received one of these formulas until 6 to 9 days of age Thereafter each infant was fed experimental formula 5018D or 5018E Each formula was prepared from fat free milk solids vegetable oils (58% corn oil 42% coconut oil) lactose vitamins and minerals Chemical composition of the formulas is presented in Table 1

At the time the infant was enrolled in the study her mother was interviewed by one of us (L N T) The program was outlined in detail and written instructions were supplied It was suggested that the infant be fed until satisfied but because caloric concentration of the formula was not discussed preconceived notions of the mother concerning volumes of intake appropriate for age may have influenced the amounts the mother attempted to feed

The experimental formula was provided in ready-to-feed disposable glass bottles containing 170 or 240 ml The entire supply of each formula (13 500 bottles) was prepared by the manufacturer as a single batch A supply of formula sufficient for 72 or 96 hours was weighed and delivered to the family When a new supply was delivered 3 or 4 days later the bottles from the previous supply including any unconsumed amounts of formula were collected and again weighed Bottles for each 24 hour period were weighed separately From the weight of formula consumed and its density volume of intake was calculated

Strained foods

During the first 18 days of life the formula and the vitamin fluoride supplement (see footnote to Table 1) served as the sole source of nutrients After 28 days of age the infants were permitted to receive commercially prepared strained foods from one manufacturer[3] according

[1] Ross Laboratories Columbus Ohio
[2] Mead Johnson and Company Evansville Indiana
[3] Gerber Products Company Fremont Michigan

INFLUENCE OF FORMULA CONCENTRATION ON CALORIC INTAKE AND GROWTH OF NORMAL INFANTS

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ABSTRACT Fomon S J Filer L J Jr Thomas L N Anderson T A and Nelson S E (Department of Pediatrics University Hospital University of Iowa Iowa City USA) Influence of formula concentration on caloric intake and growth of normal infants Acta Paediatr Scand 64 172 1975.—Fifteen fullterm female infants were enrolled in each of two feeding groups and all but one completed the proposed period of observation to age 112 days Formulas prepared from the same ingredients (fat free milk solids a mixture of corn and coconut oils lactose vitamins and minerals) were fed ad libitum to both groups Formula concentration was 54 kcal/100 ml for one group and 100 kcal/100 ml for the other A limited selection of commercially prepared strained foods was permitted after 28 days of age Weighed intakes of food were recorded for each day of study During the interval 8 through 41 days of age the infants fed the 54 kcal/100 ml formula consumed a considerably greater quantity of food but fewer calories than did those fed the 100 kcal/100 ml formula Those fed the 54 kcal/100 ml formula also gained less weight These differences between feeding groups were statistically significant After 41 days of age mean caloric intakes (kcal/kg/day) and rates of gain in weight were similar for the two feeding groups The data provide a basis for speculation on the possible difference in allocation of calories to growth and non growth in the two groups

KEY WORDS Infant feeding formula concentration food consumption growth

In industrialized societies of the Western world obesity is generally considered undesirable The association of obesity with diabetes mellitus hypertension and cardiovascular disorders is well recognized and there is evidence that at least in some societies obese persons are subjected to a subtle form of discrimination (2) Prevention of obesity may require alteration of eating habits and of current attitudes toward foods and eating

Several authors have suggested that over feeding during early infancy may give rise to an excessive population of adipocytes that then persists and predisposes the individual to subsequent obesity (1 3 8) Whether or not this occurs habits of overfeeding established in infancy may be difficult or impossible to change in later life (6*a*)

Because of the possibility that feeding during early infancy may have implications with respect to obesity during childhood and adult life it seems important to identify factors that influence food intake during infancy One such factor of importance during the early weeks of life appears to be the caloric concentration of the formula We have previously reported (4) results of studies of food intake by normal male infants fed formulas similar except for

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Table 2 *Summary of data on food intake and growth*

	Formula 5018D (54 kcal/100 ml)						Formula 5018E (100 kcal/100 ml)					
	Age interval (days)						Age interval (days)					
	8–41		42–111		8–111		8–41		42–111		8–111	
	Mean	S D	Mean	S D	Mean	S D	Mean	S D	Mean	S D	Mean	S D
Average body weight (g)	3 665	342	5 057	470	4 602	384	4 310	618	5 949	782	5 413	714
Volume of intake												
(ml/day)	735	111	939	127	873	117	540	89	582	86	568	77
(ml/kg/day)	201	31	186	21	191	23	126	17	99	10	108	8
Intake of calories												
(kcal/day)	393	59	521	74	479	66	538	90	572	81	561	75
(kcal/kg/day)	107	16	103	12	105	13	126	18	97	9	106	8
Gain in weight												
(g/day)	29 8	4 9	24 6	4 6	26 3	3 8	41 0	10 4	24 9	5 3	30 2	5 8
(g/100 kcal)	7 65	1 15	4 74	0 77	5 52	0 71	7 54	0 86	4 38	0 79	5 39	0 73
(g/mm)[a]	24 7	4 9	27 2	6 4	25 9	4 0	32 7	8 2	28 3	5 6	29 8	4 7
Gain in length (mm day)	1 23	0 17	0 92	0 15	1 02	0 11	1 26	0 17	0 88	0 13	1 01	0 09

[a] Gain in weight per unit of gain in length

day (107 kcal/kg/day) versus 538 kcal/day (126 kcal/kg/day)

During the interval 42 through 111 days of age mean quantity of food consumed was 939 ml/day (186 ml/kg/day) by infants fed Formula 5018D and 582 ml/day (99 ml/kg/day) by infants fed Formula 5018E Corresponding calorie intakes were 521 kcal/day (103 kcal/kg/day) and 572 kcal/day (97 kcal/kg/day) (Table 2) Over the entire interval 8 through 111 days of age mean calorie intake was 479 kcal/day (105 kcal/kg/day) by infants fed Formula 5018D and 561 kcal/day (106 kcal/kg/day) by infants fed Formula 5018E

Consumption of beikost (i e infant foods other than milk or formula) demonstrated wide individual variation (Appendix IV) In the age intervals 8 through 41 days and 42 through 111 days beikost accounted for approximately 2 and 12% respectively of total calorie intake by infants fed Formula 5018D and for approximately 1 and 8% respectively of total calorie intake by infants fed Formula 5018E

Gain in weight and length

As may be seen from Table 2 mean gain in weight during the interval 8 to 42 days was 29 8 g/day for infants fed the 54 kcal/100 ml formula (5018D) and 41 0 g/day for infants fed 100 kcal/100 ml formula (5018E) Corresponding mean gains in weight during the interval 42 to 112 days of age were nearly identical—24 6 and 24 9 g/day Analysis of covariance of gain in weight by feeding group adjusted for body weight at age 8 days indicates that the difference between feeding groups was statistically significant during the interval 8 to 42 days of age ($p<0\ 01$) and during the entire interval 8 to 112 days of age ($p<0\ 05$) Mean gain in weight per unit of calorie intake was similar by infants in the two feeding groups

Mean gain in length was also similar by infants in the two feeding groups (Table 2) Because gain in weight during the age interval 8 to 42 days (and also 8 to 112 days) was greater by infants fed the 100 kcal/100 ml formula (5018E) than by those fed the 54 kcal/100 ml formula (5018D) whereas gain in length was similar gain in weight per unit of gain in length was greater for infants fed 100 kcal/100 ml formula than for those fed the 54 kcal/100 ml formula Analysis of covariance of gain in weight per unit of gain in length by feeding

to the following schedule at age 28 days oatmeal with applesauce and bananas at 56 days pears and at 84 days (two foods) applesauce and bananas with tapioca The proximate composition and density of each strained food has been presented previously (5) Caloric densities of the four foods are 77 68 87 and 85 kcal per 100 g respectively Parents were advised that addition of such foods to the diet was optional and that the formula was a complete food Empty (or partially empty) strained food jars were collected at 3 or 4 day intervals and weighed From the weight of strained food consumed and its density volume of intake of each of these strained foods was calculated

Intakes of strained foods have undoubtedly been somewhat overestimated because of small losses on the bib the face of the infant and the feeding dish Percentage losses of strained foods are likely to be greatest by the youngest infants However strained food was not given during the first 28 days of life and was generally consumed in quite small quantities during the interval 28 through 111 days of age

Because significant calorie losses in the form of spilled or wasted strained foods would be likely to be reflected in weight gain per unit of calorie intake data from a previous study of 142 infants fed 67 kcal/100 ml milk based formulas (5) were analysed for the relation between percentage of calorie intake from strained food and gain in weight per unit of calorie intake Analysis of covariance of weight gain per unit of calorie intake by sex and feeding adjusted for percentage of calories from strained food failed to reveal a statistically significant difference in the interval 28 through 111 days of age

Measurements of weight and length

Body weight was determined to the nearest 5 g with infant scales [4] Length was determined as described by Fomon (6*b*) Measurements were made between 6 and 9 days of age within 2 days of each of the following ages 14 28 42 and 56 days and within 4 days of ages 84 and 112 days

INTERVALS OF STUDY

In describing size of the infants (e g Appendices I and II) recorded measurements were corrected by parabolic interpolation or extrapolation utilizing three adjacent values to obtain values applicable to ages 8 14 28 42 56 84 and 112 days For convenience these age designations have been employed throughout Gains in length and weight are expressed as 8 to 42 (8–42) 42 to 112 (42–112) or 8 to 112 (8–112) days

The day on which measurements of length and weight were made was employed as the first day of the interval for recording food intake Thus volume of intake and caloric intake are presented (Table 2) for ages 8 through 41 (8–41) 42 through 111 (42–111) or 8 through 111 (8–111) days When data on food intake concerned an interval that did not exactly conform to the designated ages—e g 15–29 days (or 15–28 days)—the actual food intake was divided by the number of elapsed days and food intake per day was then recorded for the appropriate interval (e g 14–28 days) In the case of discussions involving data both on food intake and growth the designations 8–41 42–111 or 8–111 days are used

Mean values for body weight during the intervals 8–42 and 42–112 days are employed in certain of the calculations presented in the Discussion Mean body weight for the 34-day interval from 8 to 42 days of age was estimated as follows mean weight for the interval 8–14 days was assumed to be the sum of the weight at 8 days and the weight at 14 days divided by 2 similarly the mean weight for the interval 14–28 days and for the interval 8–42 days was calculated The mean weight for the entire interval 8–42 days was then computed by weighting the values for the three intervals according to the relative durations of the intervals (i e 6 14 and 14 days respectively) Mean body weights during the intervals 42–112 days and 8–112 days were estimated in a similar manner

The method just described for estimating mean body weight was not utilized for presenting data on food intake In the case of food intake daily intakes available for the entire interval under consideration were divided by the number of elapsed days to arrive at the mean intake per day

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[4] Continental Scales Company Chicago Illinois

RESULTS

Thirty infants were enrolled 15 in each feeding group and 29 completed the planned period of observation from 8 through 111 days of age One infant (Subject 1650) was studied until 84 days of age but was then lost to further study because the family moved from town Data concerning this infant are included in Appendices I–IV but are not included elsewhere in the presentation and analysis of data

Data on weight and length of each infant at various ages are presented in Appendices I and II and data on food intake are presented in Appendices III and IV A summary of data on food intake and growth is given in Table 2

Quantity of food consumed and caloric intake

During the interval 8 through 41 days of age mean quantity of food consumed was 73 ml/day (201 ml/kg/day) by infants fed Formul 5018D and 540 ml/day (126 ml/kg/day) by in fants fed Formula 5018E (Table 2) In spite o the much greater quantity of food consume by infants fed Formula 5018D mean calori intake was considerably less by these infant than by infants fed Formula 5018E 393 kcal

Table 3 *Estimated energy utilization for growth and fat content of weight gained by infants receiving various diets in the age interval 8 through 111 days*

Formula	Formula concentration (kcal/100 ml)	Sex	Number of infants	Intake of metabolizable energy (kcal/day)	Average body weight (kg)	Energy for non growth[b] (kcal/day)	Gain in weight (g/day)	Energy for growth (kcal/day)	Energy for growth (kcal/100 g gain)	Fat in gain (%)
MBF	67	M	65	575	5 297	340	32 6	185	568	41 6
MBF	67	F	77	485	5 014	371	27 6	164	594	44 1
5018D	54	F	15	455	4 602	95	26 3	160	608	45 6
5018E	100	F	14	533	5 413	346	30 2	187	620	46 7
29C[d]	133	M	9	630	5 695	364	35 6	266	747	59 3

Assuming 95% of calorie intake is metabolizable energy
[b] Assuming 64 kcal/kg/day for non growth
Various milk based formulas studied previously (5)
[d] An experimental formula studied previously (4)

of fat free tissue will require 1 46 kcal Utilizing this value for the energy cost of synthesis of fat free tissue and 11 6 kcal per gram as the energy cost of synthesis of fat a total energy cost of 594 kcal/100 g may be calculated to indicate that fat comprised 44 1% of the gain in weight (Table 3)—a value only slightly different from the estimated percentage of fat in the gain by the male reference infant

Calculations of a similar nature indicate that during the interval 8 through 111 days of age fat accounted for 45 6 and 46 7% respectively of the gain by infants fed the 54 kcal/100 ml (5018D) and the 100 kcal/100 ml (5018E) formulas in the present study Thus these assumptions do not support the conclusion that the composition of gain in weight by the infants fed 54 or 100 kcal/100 ml formulas differed in a major way from that of infants fed 67 kcal/100 ml formulas

However calculations concerning male infants fed a 133 kcal/100 ml formula (Formula 29C) (4) suggest that fat comprised a relatively large percentage of the gain in weight—59 3% (Table 3) Therefore it seems possible that the percentage of fat in tissue gained between 8 and 112 days of age is similar when infants are fed formulas ad libitum providing all essential nutrients in a highly digestible form and caloric density of the formula does not differ more widely than 54 to 100 kcal/100 ml Clinical observations both in developed and in preindustrialized countries suggest that feedings of very low caloric density do not permit normal fat synthesis The study with a 133 kcal/100 ml formula suggests that formulas of very high caloric density may promote excessive fat synthesis

Calculations similar to those discussed in relation to Table 3 may of course be applied to shorter age intervals If one assumes 64 kcal/kg/day for non growth for all ages it is found that the energy cost of tissue synthesis is less for the interval 8 through 41 days or 8 through 55 days than for the subsequent interval through 111 days The difference is exaggerated with respect to infants fed the 54 kcal/100 ml formula (5018D) During the interval 8 through 41 days of age the average energy cost of growth is calculated to be 463 kcal/100 g (equivalent to 31 3% fat) whereas during the interval 42 through 111 days of age the energy cost of growth is calculated to be 695 kcal/100 g (equivalent to 54 1% fat) Corresponding values for infants fed the 100 kcal/100 ml formula (5018E) are 573 and 651 kcal/100 g equivalent to 42 1 and 49 8% fat in the two age intervals

Even if caloric expenditures for non growth average 64 kcal/kg/day over the entire interval 8 through 111 days of age there is little reason to assume that caloric expenditures for non growth are identical per unit of body weight for younger and older infants It seems likely

group adjusted for body weight at age 8 days indicates that the difference between feeding groups was statistically significant during the intervals 8 to 42 days of age ($p<0.01$) and 8 to 112 days of age ($p<0.05$)

DISCUSSION

In the interval 8 through 41 days of age mean calorie intake whether expressed as kcal/day or kcal/kg/day was significantly less by infants fed the 54 kcal/100 ml formula than by those fed the 100 kcal/100 ml formula Several hypotheses may be offered to explain the difference in mean caloric intake between the two feeding groups Volumes of food[5] consumed by infants fed the 54 kcal/100 ml formula may approach the volume capacity of the infants so that larger volumes and hence larger calorie intakes could not be achieved Conversely in the case of infants fed the 100 kcal/100 ml formula it is possible that during the early weeks of life some minimum volume of food must be consumed in order to satisfy the infant In addition to these possibilities or as an alternative it may be that preconceived notions of parents or other caretakers regarding usual or desirable volumes of intake were responsible for modifying the volume of food that would have been consumed with these feedings if the infant had been permitted to feed without the mother s knowledge of the quantity consumed as for example in breast feeding It is apparent that ad libitum feeding must be interpreted differently in breast and bottle feeding

Not only was calorie intake greater by infants fed the 100 kcal/100 ml formula but gain in weight was greater One therefore wonders whether the greater gain in weight associated with greater caloric intakes results in a different composition of gain—e g a greater fat content per unit of gain in weight

[5] During the age interval 8 through 27 days of age only formula was consumed From 28 through 41 days of age some infants received in addition wet packed strained oatmeal with applesauce and bananas (Appendix IV)

In a previous publication (5), we speculated about the allocation of caloric intake by male infants to growth and non growth The speculations presented here are based on assumptions modified only slightly from those employed previously (1) 95% of caloric intake is available as metabolizable energy (2) The energy cost of tissue synthesis is adequately described by energy required for synthesizing protein and fat (3) The energy cost of synthesis of protein is 7 5 kcal/g and that of fat is 11 6 kcal/g—values reported by Kielanowski (7) from studies of growing pigs (4) During the interval 8 through 111 days of age composition of the gain in weight by male infants fed milk based formulas as previously reported (5) is the same as that attributed to the male reference infant (6c) between birth and 4 months of age 11 4% protein and 41 6% fat (5) Mean energy expenditures for non growth are the same per unit of body weight by males and females receiving a variety of feedings

On the basis of the preceding assumptions the intake of metabolizable energy during the interval 8 through 111 days of age by male infants fed 67 kcal/100 ml milk based formulas (5) averaged 525 kcal/day and energy for growth averaged 185 kcal/day (Table 3) Therefore energy expenditures for non growth averaged 340 kcal/day or for an average body weight of 5 292 kg 64 kcal/kg/day

On the assumption that caloric expenditures for non growth are the same per unit of body weight for females as for males and for various feedings it may be calculated that for female infants fed 67 kcal/100 ml milk based formulas in the previous study (5) energy expenditures for non growth averaged 321 kcal/day and energy available for growth (intake of metabolizable energy minus energy for non growth) averaged 164 kcal/day or 594 kcal/100 g gain (Table 3)

Employing the assumptions previously stated that 7 5 kcal are required for synthesis of one gram of protein and that gain in weight in the interval 8 through 111 days is 58 4% fat free (i e 41 6% fat) synthesis of one gram

Table 3 *Estimated energy utilization for growth and fat content of weight gained by infants receiving various diets in the age interval 8 through 111 days*

Formula	Formula concentration (kcal/100 ml)	Sex	Number of infants	Intake of metabolizable energy (kcal/day)	Average body weight (kg)	Energy for non growth[b] (kcal/day)	Gain in weight (g/day)	Energy for growth (kcal/day)	Energy for growth (kcal/100 g gain)	Fat in gain (%)
MBF	67	M	65	525	5 292	340	32 6	185	568	41 6
MBF	67	F	77	485	5 014	3 1	27 6	164	594	44 1
5018D	54	F	15	455	4 602	295	6 3	160	608	45 6
5018E	100	F	14	533	5 413	346	30 2	187	6 0	46 7
29C[d]	133	M	9	630	5 695	364	35 6	266	747	59 3

[a] Assuming 95% of calorie intake is metabolizable energy
[b] Assuming 64 kcal/kg/day for non growth
[c] Various milk based formulas studied previously (5)
[d] An experimental formula studied previously (4)

of fat free tissue will require 1 46 kcal Utiliz ing this value for the energy cost of synthesis of fat free tissue and 11 6 kcal per gram as the energy cost of synthesis of fa a total energy cost of 594 kcal/100 g may be calculated to indicate that fat comprised 44 1% of the gain in weight (Table 3)—a value only slightly dif ferent from the estimated percentage of fat in the gain by the male reference infant

Calculations of a similar nature indicate that during the interval 8 through 111 days of age fat accounted for 45 6 and 46 7% respective ly of the gain by infants fed the 54 kcal/100 ml (5018D) and the 100 kcal/100 ml (5018E) formulas in the present study Thus these as sumptions do not support the conclusion that the composition of gain in weight by the in fants fed 54 or 100 kcal/100 ml formulas dif fered in a major way from that of infants fed 67 kcal/100 ml formulas

However calculations concerning male in fants fed a 133 kcal/100 ml formula (Formula 29C) (4) suggest that fat comprised a rela tively large percentage of the gain in weight—59 3% (Table 3) Therefore it seems possible that the percentage of fat in tissue gained between 8 and 112 days of age is similar when infants are fed formulas ad libitum pro viding all essential nutrients in a highly di gestible form and caloric density of the formula does not differ more widely than 54 to 100 kcal/100 ml Clinical observations both in developed and in preindustrialized countries suggest that feedings of very low caloric den sity do not permit normal fat synthesis The study with a 133 kcal/100 ml formula suggests that formulas of very high caloric density may promote excessive fat synthesis

Calculations similar to those discussed in relation to Table 3 may of course be applied to shorter age intervals If one assumes 64 kcal/kg/day for non growth for all ages it is found that the energy cost of tissue synthesis is less for the interval 8 through 41 days or 8 through 55 days than for the subsequent inter val through 111 days The difference is exag gerated with respect to infants fed the 54 kcal/100 ml formula (5018D) During the inter val 8 through 41 days of age the average energy cost of growth is calculated to be 463 kcal/100 g (equivalent to 31 3% fat) whereas during the interval 42 through 111 days of age the energy cost of growth is cal culated to be 695 kcal/100 g (equivalent to 54 1% fat) Corresponding values for infants fed the 100 kcal/100 ml formula (5018E) are 573 and 651 kcal/100 g equivalent to 42 1 and 49 8% fat in the two age intervals

Even if caloric expenditures for non growth average 64 kcal/kg/day over the entire interval 8 through 111 days of age there is little reason to assume that caloric expenditures for non growth are identical per unit of body weight for younger and older infants It seems likely

that older infants are more active than younger infants and therefore caloric expenditures for non growth might be less than 64 kcal/kg/day during the interval 8 through 41 days of age and more than 64 kcal/kg/day during the interval 42 through 111 days of age Further the efficiency of synthesis of protein and fat might conceivably be greater for younger than for older infants One can only conclude that energy relationships are different during the interval 8 through 41 days of age than during the interval 42 through 111 days of age The differences could be explained by a lesser percentage of fat in the gain by the younger infants a lesser energy expenditure for non growth per unit of body weight and/or a more efficient synthesis of fat (and possibly protein) by younger than by older infants Studies other than the type reported here will be necessary to distinguish between these possibilities

Although the calculations summarized in Table 3 suggest that the percentage of fat in weight gained by infants fed Formulas 5018D and 5018E were similar in the interval 8 through 111 days of age similar calculations suggest that in the interval 8 through 41 days of age fat comprised a greater percentage of the gain of infants fed the formula with greater caloric density (Formula 5018E)

From the preceding discussion it is evident that a great deal of additional information is necessary to achieve an adequate understanding of the relationships between caloric intake and growth The most important single bit of information missing in the present studies is an independent estimate of caloric expenditures for non growth In the future it is hoped that such data may be obtained

REFERENCES

1 Brook C G D Lloyd J K & Wolf O H Relation between age of onset of obesity and size and number of adipose cells *Br Med J II* 25 1972

2 Canning H & Mayer J Obesity—its possible effect on college acceptance *N Engl J Med 275* 1177 1966

3 Editorial Infant and adult obesity *Lancet I* 17 1974

4 Fomon S J Filer L J Jr Thomas L N Rogers R R & Proksch A M Relationship between formula concentration and rate of growth of normal infants *J Nutr 98* 241 1969

5 Fomon S J Thomas L N Filer L J Jr Ziegler E E & Leonard M T Food consumption and growth of normal infants fed milk based formulas *Acta Paediatr Scand* Suppl *223* 1971

6 Fomon S J *Infant nutrition* W B Saunders Company Philadelphia 1974 (*a*) p 22 (*b*) p 35 (*c*) p 71

7 Kielanowski J Estimates of the energy cost of protein deposition in growing animals *In* Blaxter K L (ed) *Proceedings of Third Symposium on Energy Metabolism* Academic Press New York 1969 p 13

8 Knittle J L Obesity in childhood A problem in adipose tissue cellular development *J Pediatr 81* 1048 1972

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APPENDIX I

Weights (g) of Individual Female Infants at Various Ages

Subject number	Birth	8 days	14 days	28 days	4? days	56 days	84 days	112 days
Formula 5018D								
16?1	3 ?10	3 127	3 29?	3 635	4 001	4 363	5 ?67	6 136
16 ?	3 000	3 076	3 ?25	3 712	4 075	4 450	5 085	5 687
16 3	3 ?60	3 097	3 ?44	3 714	4 16?	4 598	5 ?58	5 787
16 4	730	2 58?	? 804	3 258	3 758	4 ?16	4 880	5 613
1625	3 7?0	3 570	3 625	4 100	4 460	5 008	5 555	6 455
16?6	3 170	2 916	3 098	3 614	4 105	4 664	5 447	6 23?
16 7	900	2 968	3 100	3 531	3 875	4 217	4 8?0	5 47?
16 8	3 080	3 000	3 081	3 493	3 871	4 3?9	5 094	6 017
16?9	2 940	3 100	3 319	3 808	4 ?45	4 648	5 349	5 979
1630	? 780	2 717	2 835	3 200	3 480	3 7?5	4 250	4 550
1631	3 080	3 040	3 34?	3 731	4 033	4 364	4 779	5 ?65
163?	3 8 0	3 8?0	3 980	4 2?7	4 86?	5 191	5 965	6 869
1633	3 350	3 310	3 347	3 966	4 385	4 810	5 231	5 901
1634	3 9?0	3 860	3 875	4 3?0	4 680	4 905	5 556	5 9?6
1635	3 300	3 ?67	3 480	4 100	4 650	5 150	5 710	6 ?84
Formula 5018E								
1636	2 9?0	? 95	3 ?50	4 ?30	4 890	5 520	6 205	6 65?
1637	3 170	3 ?80	3 554	4 09	4 715	5 ?04	5 940	6 597
1638	3 110	3 175	3 366	3 907	4 4?3	4 797	5 666	6 164
1639	3 0?0	2 925	3 008	3 414	3 812	4 037	4 ?07	5 005
1640	4 465	4 359	4 571	5 1?3	5 676	5 751	6 390	6 808
1641	3 8?0	3 977	4 190	5 050	5 850	6 500	7 438	7 975
164?	? 8?0	? 825	? 959	3 441	3 891	4 254	4 99?	5 694
1643	3 870	3 957	4 361	5 108	5 755	6 169	6 755	7 190
1644	4 730	4 695	4 838	5 484	5 885	6 467	7 19?	7 991
1645	3 270	3 ? 9	3 460	4 375	5 080	5 575	6 758	7 468
1646	3 240	3 30?	3 525	4 180	4 680	4 9?5	5 550	6 0?0
164	4 340	4 ?00	4 387	4 841	5 ?98	5 561	6 204	6 897
1648	3 970	3 934	4 160	4 8?0	5 4?5	5 960	6 855	7 500
1649	3 850	3 817	4 015	4 130	4 765	5 ?81	5 953	6 594
1650	3 630	3 6?0	3 844	4 301	4 666	4 978	5 547	

APPENDIX II

Lengths (cm) of Individual Female Infants at Various Ages

Subject number	Birth date	8 days	14 days	8 days	4? days	56 days	84 days	11? days
Formula 5018D								
16 1	31/7/71	49 0	49 3	50 9	5? 5	53 6	56 6	59 0
16	6/8/71	48 8	49 6	50 8	5? 8	54 6	57 3	59 4
16 3	?5/8/71	50 1	50 7	5? 1	54 1	55 3	57 8	60 6
16?4	13/9/71	46 8	47 9	49 8	51 1	5? 5	55 3	58 7
16?5	9/9/71	51 7	5 8	55 0	57 0	57 2	59 2	61 5
16 6	3/10/71	49 0	49 8	51 5	5? 9	55 3	57 7	60 ?
16?7	9/10/71	49 3	50 2	5? 3	54 2	55 8	58 ?	61 5
16 8	31/10/71	49 6	50 4	5 3	54 1	55 6	58 8	6? 7
16 9	25/11/71	50 8	51 1	5 1	55 2	57 5	59 1	60 9
1630	?1/1/7?	47	48 ?	50 9	51 8	53 4	55 6	57 4
1631	?0/1/72	49 ?	50 4	5 0	53 0	53 8	55 5	57 7
163	30/1/7?	51 2	5? 4	53 5	54 4	56 4	59 3	60 9
163?	10/?/7?	50 5	51 4	5 ?	54 4	56 0	59 3	61 3
1634	6/3/7?	51 5	5 1	53 3	54 9	56 0	58 4	61 0
1635	8/3/72	51 1	51 8	53 8	55 8	57 1	59 3	6? 4

Continued on p 180

Appendix II (cont)

Subject number	Birth date	8 days	14 days	28 days	42 days	56 days	84 days	112 days
Formula 5018E								
1636	28/7/71	49.6	50.6	53.3	54.1	55.7	58.4	59.9
1637	5/8/71	50.7	51.5	53.2	54.5	56.3	58.8	61.4
1638	19/8/71	49.5	50.4	52.6	54.2	55.6	59.2	60.8
1639	4/9/71	48.9	49.3	50.1	52.4	54.1	57.3	57.9
1640	27/9/71	52.2	53.3	55.1	57.2	57.6	60.0	61.4
1641	8/10/71	53.5	54.8	56.1	57.4	59.4	62.7	65.6
1642	17/10/71	49.2	49.5	52.0	53.9	54.9	57.6	60.0
1643	1/11/71	56.2	56.8	59.4	60.9	62.3	63.6	67.7
1644	16/11/71	54.6	55.5	57.8	59.9	61.0	63.6	66.2
1645	1/12/71	49.4	50.9	52.1	54.0	54.7	58.1	59.7
1646	17/12/71	50.2	51.5	53.2	54.3	55.7	58.9	60.7
1647	24/1/72	53.5	53.8	55.5	56.8	58.6	59.9	62.6
1648	4/2/72	52.7	53.0	55.0	56.8	58.6	60.3	63.5
1649	14/2/72	55.0	55.5	57.1	58.9	60.3	62.3	64.6
1650	28/3/72	51.3	51.4	53.9	55.1	58.0	58.7	

APPENDIX III

Average Daily Volume (ml) of Formula Consumed by Individual Female Infants During Successive Age Intervals

Subject number	Age interval (days)					
	8–13	14–27	28–41	42–55	56–83	84–111
Formula 5018D						
1621	544	750	765	816	924	975
1622	584	674	738	788	816	908
1623	513	658	663	695	728	714
1624	431	594	805	791	795	952
1625	447	552	586	753	887	1 021
1626	637	1 050	1 151	1 158	1 203	1 241
1627	559	715	768	738	791	972
1628	473	650	682	751	815	746
1629	705	710	828	899	839	920
1630	460	574	665	685	732	727
1631	666	755	796	856	667	906
1632	623	742	807	840	848	751
1633	651	806	879	838	706	1 087
1634	592	760	896	879	930	938
1635	661	818	864	835	682	822
Formula 5018E						
1636	466	664	707	658	586	554
1637	484	573	530	538	487	518
1638	432	489	503	485	439	460
1639	295	409	382	407	474	431
1640	561	564	486	417	544	662
1641	510	662	718	771	655	633
1642	319	417	442	471	504	526
1643	590	690	703	608	508	390
1644	407	540	583	581	642	657
1645	428	579	688	506	699	544
1646	426	527	542	442	464	449
1647	456	482	487	453	463	540
1648	499	569	590	593	573	580
1649	419	365	466	472	429	437
1650	486	469	409	369	367	

APPENDIX IV

Average Daily Weight (g) of Strained Foods Consumed by Individual Female Infants

Subject number	Oatmeal with bananas and applesauce				Pears		Applesauce	Bananas
	28–41	4?–55	?6–83	84–111	56–83	84–111	84–111	84–111
Formula 5018D								
16?1	1	4	10	?2	6	26		14
16??				20		15	16	15
16?3			85	70	89	60	19	44
16 4	40	65	37	30	17			22
1625	57	46	58	46	2		2	4
16?6		3?	45	107	39	60	?6	39
16?7	16	5?	34	18	31	18		8
16 8	10?	81	69	98	68	30	11	14
16 9	18	21	27	36	24	18	14	23
1630			7	50		4		
1631	68	64	53	62	24	?4	?8	10
163?	66	103	68	102	83	3?	40	43
1633	10	48	24		15	10	5	
1634	?5	5?	64	46	15	15	14	28
1635	38	107	76	101	89	36	?3	17
Formula 5018E								
1636	4	14	2	4	1	3		?
1637	9	18	45	?1	11	3	5	8
1638	16	19	67	9?	48	49	0	5
1639	16	18	7	14	11	5	5	6
1640	69	108	213	14?	1?	5	39	72
1641	13	1		25		16		2
164?								
1643	9	6	29	34	63	5?		34
1644								
1645	15	33	?4	?8				14
1646								
1647		4	65	17	2?	24	10	4
1648				10		2	2	2
1649	160	77	12?	138	67	28	35	37
16?0	56	71	51		55			

Appendix II (cont.)

Subject number	Birth date	8 days	14 days	28 days	42 days	56 days	84 days	112 days
Formula 5018F								
1636	28/7/71	49 6	50 6	53 3	54 1	55 7	58 4	59 9
1637	5/8/71	50 7	51 5	53 2	54 5	56 3	58 8	61 4
1638	19/8/71	49 5	50 4	52 6	54 2	55 6	59 2	60 8
1639	4/9/71	48 9	49 3	50 1	52 4	54 1	57 3	57 9
1640	27/9/71	52 2	53 3	55 1	57 2	57 6	60 0	61 4
1641	8/10/71	53 5	54 8	56 1	57 4	59 4	62 7	65 6
1642	17/10/71	49 2	49 5	52 0	53 9	54 9	57 6	60 0
1643	1/11/71	56 2	56 8	59 4	60 9	62 3	63 6	67 7
1644	16/11/71	54 6	55 5	57 8	59 9	61 0	63 6	66 2
1645	1/12/71	49 4	50 9	52 1	54 0	54 7	58 1	59 7
1646	17/12/71	50 2	51 5	53 2	54 3	55 7	58 9	60 7
1647	24/1/72	53 5	53 8	55 5	56 8	58 6	59 9	62 6
1648	4/2/72	52 7	53 0	55 0	56 8	58 6	60 3	63 5
1649	14/2/72	55 0	55 5	57 1	58 9	60 3	62 3	64 6
1650	28/3/72	51 3	51 4	53 9	55 1	58 0	58 7	

APPENDIX III

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Subject number	Age interval (days)					
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1622	584	674	738	788	816	908
1623	513	658	663	695	728	714
1624	431	594	805	791	795	952
1625	447	552	586	753	887	1 021
1626	637	1 050	1 151	1 158	1 203	1 241
1627	559	715	768	738	791	922
1628	473	650	682	751	815	746
1629	705	710	828	899	839	920
1630	460	574	665	685	732	727
1631	666	755	796	856	667	906
1632	623	747	807	840	848	751
1633	651	806	879	838	706	1 087
1634	592	760	896	879	930	938
1635	661	818	864	835	682	822
Formula 5018E						
1636	466	664	707	658	586	554
1637	484	573	530	538	487	518
1638	432	489	503	485	439	460
1639	295	409	382	407	424	431
1640	561	564	486	417	544	662
1641	510	662	718	771	655	633
1642	319	417	442	471	504	526
1643	590	690	703	608	508	390
1644	407	540	583	581	642	657
1645	478	579	688	506	699	544
1646	426	527	542	442	464	449
1647	456	482	487	453	463	540
1648	499	569	590	593	573	580
1649	419	365	466	472	429	437
1650	486	469	409	369	367	

males and 3 males aged 6 months to ?4 years with an average age of 6 $^{8}/_{12}$ years The diagnosis with regard to the site of infection was based on measuring the rectal temperature the micro erythrocyte sedimentation rate (ESR) the renal concentrating capacity and the titre of *E coli* antibody As abnormal values of these items were considered a temperature of 38°C or more an ESR of at least 20 mm per hour a renal concentrating capacity of less than 800 mosm/kg water and a fourfold or greater rise in *E coli* antibody titre to at least 256 The infection was considered to be a pyelonephritis when at least three of these four items were pathological When none of the items were abnormal and the clinical symptoms were micturition abnormalities only the patients were classified as having cystitis

According to these criteria the patients were separated into three groups

Group 1 8 patients with pyelonephritis

Group 2 11 patients with cystitis and 5 patients with ABU

Group 3 13 patients who could not be classified with in group 1 or ? The records of these 13 patients were scrutinized without knowledge of the results of the NBT tests and the patients were classified according to their symptoms and laboratory findings in two subgroups 3 A 6 patients with probable pyelonephritis and group 3 B 7 patients with probable cystitis or ABU

The patients received *appropriate* chemotherapy usually sulphonamide for 10 days and were readmitted to the hospital after 2 to 10 weeks for a clinical and laboratory check up with urine culture urinary cell count NBT test ESR renal concentrating capacity and titration of antibodies against *E coli*

METHODS

Urine specimens for *culture* were saved after thorough cleansing of the external genitalia with soap and water When possible a mid stream portion was used The urine specimens were immediately chilled to 4 C and kept at this temperature until culture was performed This was done with a calibrated loop technique and the *E coli* isolates were submitted to simplified 0-grouping (13)

The blood leucocytes were counted in capillary blood The number of neutrophils was obtained by multiplying the total white blood cell count with the percentage of neutrophilic granulocytes or by counting the number of polymorphonuclear cells in a Burker chamber

The urinary leucocytes were counted in uncentrifuged fresh urine in a Fuchs Rosenthal chamber The osmolality of urine was determined after 10–14 hours water deprivation If the osmolality of urine was less than 800 mosm/kg water the renal concentrating capacity was determined after water deprivation for 16–17 hours and administration of pitressin tannate as described by Winberg (17)

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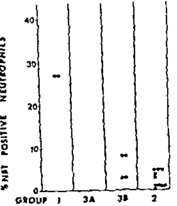


Fig 1 Percentage of NBT positive neutrophils in 8 patients with pyelonephritis (group 1) 6 with probable pyelonephritis (group 3 A) 7 with probable cystitis or ABU (group 3 B) and 16 with cystitis or ABU (group 2)

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The NBT tests and endotoxin stimulated NBT tests were performed within 6 hours of drawing the blood samples and as described elsewhere (5) using venous blood with 10–?0 IU heparin (Vitrum AB Stockholm) added per ml of blood The samples were incubated with a 0 1% solution of NBT in saline in a 37 C water bath for 25 minutes and smears were made on glass slides and counterstained with Giemsa stain One hundred neutrophils were counted and the percentage of neutrophils containing intracellular deposits of forma

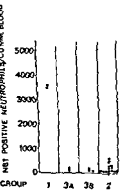


Fig 2 Number of NBT-positive neutrophils expressed in thousands per mm blood in 8 patients with pyelonephritis (group 1) 5 with probable pyelonephritis (group 3 A) 7 with probable cystitis or ABU (group 3 B) and 14 with cystitis or ABU (group 2)

Acta Pædiatr Scand 64 182–186 1975

USE OF THE NITROBLUE TETRAZOLIUM (NBT) TEST IN THE DIFFERENTIATION BETWEEN PYELONEPHRITIS AND CYSTITIS

B BJÖRKSTÉN and P de CHATEAU

From the Department of Paediatrics Umeå University Umeå Sweden

ABSTRACT Bjorkstén B and de Chateau P (Department of Paediatrics Umeå University Umeå Sweden) Use of the Nitroblue Tetrazolium (NBT) Test in the differentiation between pyelonephritis and cystitis Acta Paediatr Scand 64 182 1975 — NBT tests were performed on blood from 37 patients with urinary tract infections caused by coliform bacteria The level of infections was evaluated by the clinical signs ESR renal concentrating capacity and titre of antibodies against the strains of *E coli* isolated in their urine Elevated proportions of NBT positive neutrophils were found in 11 of 14 patients with pyelonephritis and in 3 of 23 patients with cystitis or asymptomatic bacteriuria (ABU) The total number of NBT positive neutrophils was 1 000 or more per mm³ blood in 11 of 13 patients considered to have pyelonephritis while it was 800 or less in all the patients investigated evaluated as having cystitis or asymptomatic bacteriuria The NBT test is recommended as an adjunct in the level diagnosis or urinary tract infections in children The utility of the test in smouldering pyelonephritis is presently being investigated

KEY WORDS Nitroblue tetrazolium test pyelonephritis cystitis level diagnosis in urinary tract infection

The differentiation of lower urinary tract infections from pyelonephritis in childhood is difficult when based solely on clinical symptoms In addition to fever loin pain elevated ESR lowered renal concentrating capacity and elevated titres of *E coli* antibodies indicate pyelonephritis (2) Since evaluation of the last two parameters is time consuming and the methods are not always available there is an obvious need for other methods for level differentiation

By the Nitroblue Tetrazolium (NBT) Test introduced by Park et al in 1968 (14) neutrophilic granulocytes can be divided into NBT positive and NBT negative depending on whether they reduce the dye into nitro blue formazan or not The test has been recommended as an aid in the differentiation of bacterial fungal and parasitic infections from other febrile conditions (1 8 15) After standardizing the test method however we found that it is of limited value in the differentiation of streptococcal and non streptococcal throat infections (7) and in the diagnosis of perforated appendicitis (8)

The aim of this study was to investigate whether the NBT test could be used as an aid in the differentiation of pyelonephritis from urinary tract infections not involving the renal parenchyma

MATERIAL

All patients admitted to the study had at least one bacterial count of 100000 coliform microorganisms or more per ml of urine The patients with asymptomatic bacteriuria (ABU) had several positive urine cultures (6 patients) or had one positive urinary culture and pyuria of at least 170 white cells per mm³ uncentrifugated urine (? patients) The study included 34 fe

males and 3 males aged 6 months to ?4 years with an average age of 6 ⁸/₁₂ years The diagnosis with regard to the site of infection was based on measuring the rectal temperature the micro erythrocyte sedimentation rate (ESR) the renal concentrating capacity and the titre of *E coli* antibody As abnormal values of these items were considered a temperature of 38 C or more an ESR of at least 20 mm per hour a renal concentrating capacity of less than 800 mosm/kg water and a four fold or greater rise in *E coli* antibody titre to at least 256 The infection was considered to be a pyelonephritis when at least three of these four items were pathological When none of the items were abnormal and the clinical symptoms were micturition abnormalities only the patients were classified as having cystitis

According to these criteria the patients were separated into three groups

Group 1 8 patients with pyelonephritis

Group 2 11 patients with cystitis and 5 patients with ABU

Group 3 13 patients who could not be classified within group 1 or 2 The records of these 13 patients were scrutinized without knowledge of the results of the NBT tests and the patients were classified according to their symptoms and laboratory findings in two subgroups 3 A 6 patients with probable pyelonephritis and group 3 B 7 patients with probable cystitis or ABU

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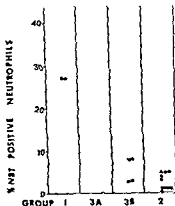


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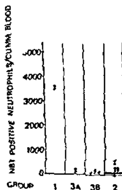


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Table 1 *Some clinical data on and laboratory findings in 37 patients with urinary tract infection*

Pat no	Age	Type of infection[a]	White cells in blood per mm³	Neutrophils in blood per mm³	NBT positive neutrophils %	NBT positive neutrophils per mm³
Group 1 Pyelonephritis (8)						
1	7	Rec	15 600	12 800	27	3 500
2	7/12	1st	21 300	15 900	26	4 100
3	10/12	1st	23 000	18 900	27	5 100
4	8/12	1st	10 200	6 100	37	2 400
5	10/12	1st	16 000	11 200	32	3 600
6	6/12	1st	13 500	9 500	41	3 900
7	6/12	Rec	21 100	16 800	10	1 700
8	1 6/12	Rec	10 400	6 300	16	1 000
Group 3 A Probable pyelonephritis (6)						
9	9/12	Rec	17 600	8 200	34	2 800
10	10	1st	6 300	3 200	4	100
11	6/12	1st	12 600	6 200	29	1 800
12	22 8/12	Rec	ND	ND	36	ND
13	10	Rec	5 100	2 600	6	200
14	6/12	1st	10 000	4 600	30	1 400
Group 2 Cystitis (11) and ABU (5)						
15	4 6/12	Rec ABU	6 900	2 600	23	600
16	6 2/12	Rec ABU	7 200	3 100	5	200
17	7 8/12	Rec	4 600	2 300	5	100
18	24 2/12	Rec	6 800	2 700	12	300
19	20 1/12	Rec	12 700	9 700	3	300
20	1	Rec ABU	6 400	1 600	6	100
21	12 3/12	Rec	4 100	ND	1	ND
22	6 6/12	1st	5 200	1 500	4	100
23	9 1/12	Rec	6 900	4 200	1	100
24	9 4/12	1st ABU	6 300	2 200	21	500
25	5 9/12	1st	ND	ND	5	ND
26	10	Rec	5 400	2 800	8	200
27	11 7/12	1st	12 400	10 400	1	100
28	9 2/12	Rec ABU	6 800	4 100	1	100
29	2 2/12	Rec	5 100	700	0	0
30	6 2/12	Rec	3 900	1 200	2	100
Group 3 B Probable cystitis (4) and probable ABU (3)						
31	8 10/12	1st	6 500	5 100	8	400
32	12 2/12	Rec	14 100	10 300	6	600
33	14 8/12	Rec	6 600	3 400	3	100
34	9 1/12	Rec ABU	7 300	4 100	2	100
35	5 7/12	Rec	4 000	2 100	8	200
36	2 5/12	Rec ABU	5 500	2 400	32	800
37	4 7/12	Rec ABU	5 500	3 300	3	100

[a] Type of infection 1st or recurrent (rec) asymptomatic bacteriuria (ABU) ND=not done

zan NBT positive cells were recorded With this method normally less than 13% of the neutrophils are NBT positive (7) The total number of NBT positive neutrophils was obtained by multiplying the number of neutrophils per mm³ blood by the percentage of NBT positive neutrophils This number is not normally more than 500 per mm³ blood in healthy children (6)

For statistical evaluation of the results the ranking test for unpaired measurements developed by Wilcoxon was used (16)

RESULTS

The NBT test results are given in Figs 1 and 2 and Table 1 The proportions of NBT positive neutrophils were elevated in 11 of the 14 patients with a definite or probable pyelonephritis (Table 1 Fig 1) Of the 23 patients with definite or probable cystitis

or ABU 3 patients with ABU had an elevated proportion of NBT positive neutrophils The total number of NBT positive neutrophils was at least 1000 cells per mm³ blood in 11 of 13 patients with definite or probable pyelonephritis while it was 800 or less in all the 21 patients investigated with definite or probable cystitis or ABU (Table 1 Fig 2)

The total white blood cell and neutrophil counts were significantly higher ($p<0.01$) in the patients with pyelonephritis than in those with cystitis or ABU (Table 1)

At the time of check up the patients had no clinical symptoms suggestive of urinary tract infection and bacterial urine cultures were negative

DISCUSSION

This study shows that in urinary tract infections the NBT test confirms a level diagnosis based on clinical symptoms ESR renal concentrating capacity and *E coli* antibody titres However a few inconsistencies were observed One patient (no 7 in Table 1) with pyelonephritis according to the criteria used had a normal proportion of NBT positive neutrophils in the blood but in this patient the total number was higher than in any of the patients with cystitis In 2 other patients where both the proportion and the total number of NBT positive neutrophils were normal the results were difficult to evaluate Endotoxin stimulated NBT test results of 52 and 55% respectively would exclude the possibility of non responsiveness of their neutrophils These 2 patients had a fever of 38 8 and 38 2°C and an ESR of 55 and 32 mm per hour respectively but the urine concentrating capacity was within normal limits (urine osmolality 900 mosm/kg water) and they had normal *E coli* antibody titres Whether the normal NBT test results obtained in these patients represent failure of the NBT test method or not is open to discussion Fever and high ESR would indicate pyelonephritis while normal *E coli* antibody titre and normal renal concentrating capacity are consistent with an infection not involving the renal parenchyma Such discrepancies between the expected and the actual results are observed with all methods used for level diagnosis including the wash out procedures (11)

Three patients with ABU had elevated NBT test values Since an ABU may be associated with smouldering pyelonephritis (for references see 4) renal involvement in these patients cannot be excluded

The number of neutrophils was higher in the patients with pyelonephritis than in those with cystitis (Table 1) and as seen in Figs 1 and 2 the overlap in NBT test results between the different groups of patients was less when the total number instead of the proportion of NBT positive neutrophils was evaluated

The performance of the NBT test must be carefully standardized since many technical factors influence on the test (5) Even with a standardized technique however normal NBT test results may be encountered in bacterial infections on the other hand elevated NBT test figures may be found in patients without a bacterial fungal or parasitic infection (6)

Thus the value of the NBT test in clinical practice is not settled and further work is needed in order to evaluate the use of the test in different well-defined clinical conditions

This investigation indicates that the NBT test is of value in the level diagnosis of urinary tract infections Fever in patients with urinary tract infection is considered to indicate pyelonephritis (2 17) and thus there is no obvious need for using the NBT test to support the diagnosis in these patients Pyelonephritis is however not always accompanied by fever Kunin et al (12) found that 13% of patients with ABU had renal scar formation indicating pyelonephritis The diagnosis of such smouldering pyelo

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nephritis is difficult and if elevated NBT test figures are encountered in these patients the NBT test would be of great clinical value We are presently studying this

REFERENCES

1 Leading article Nitroblue tetrazolium a routine test? *Lancet II* 909 1971
2 Andersen H J Hanson L Å Lincoln K Örskov I Örskov F & Winberg J Studies of urinary tract infections in infancy and childhood IV Relation of the coli antibody titre to clinical picture and to serological type of the infecting *Escherichia coli* in acute uncomplicated urinary tract infections *Acta Paediatr Scand 54* 247 1965
3 Andersen H J Studies of urinary tract infections in infancy and childhood IX Determination of *E coli* antibodies by a polyvalent antigen *Acta Paediatr Scand 56* 637 1967
4 Andersen H J Jacobsson B Larsson H & Winberg J Hypertension asymmetric renal parenchymal defect sterile urine and high *E coli* antibody titre *Br Med J III* 14 1973
5 Bjorkstén B The influence of technical factors on the NBT test *Scand J Haematol 12* 46 1974
6 — The Nitroblue tetrazolium (NBT) test A methodological and clinical study *Umeå University Medical Dissertations* No *15* 1974
7 Bjorkstén B Ekstrand T Gothefors L & Ostberg Y The Nitroblue tetrazolium (NBT) test and white blood cell count in acute throat infections *Scand J Infect Dis* In press
8 Bjorkstén B & Wahlby L The Nitroblue tetrazolium (NBT) test and white blood cell count in patients with acute abdominal pain with special reference to acute appendicitis *Acta Chir Scand* In press 1974
9 Feigin R D NBT test in the diagnosis of febrile patients *N Engl J Med 285* 347 1971
10 Holmgren J The antibody response in Rabbits to *E coli* 0 antigen *Int Arch Allergy Appl Immunol 37* 546 1970
11 Jodal U Lindberg U & Lincoln K Level diagnosis in symptomatic urinary tract infection in childhood *Acta Paediatr Scand* in press
12 Kunin C M Deutscher R & Paquin A Urinary tract infection in school children an epidemiologic clinical and laboratory study *Medicine 43* 91 1964
13 Lincoln K Lidin Janson G & Winberg J Resistant urinary infections resulting from changes in resistance pattern of faecal flora induced by sulphonamide and hospital environment *Br Med J III* 305 1970
14 Park B H Fikrig S M & Smithwick E M Infection and nitroblue tetrazolium reduction by neutrophils A diagnostic aid *Lancet II* 532 1968
15 Park B H The use and limitations of the nitroblue tetrazolium test as a diagnostic aid *J Pediatr 78* 376 1971
16 Snedecor G W & Cochran W G *Statistical methods* The Iowa State University Press Ames Iowa 1967 6th ed p 130
17 Winberg J Determination of renal concentrating capacity in infants and children without renal disease *Acta Paediatr Scand 48* 318 1959
18 Winberg J Andersen H J Hansson L Å & Lincoln K Studies of urinary tract infections in infancy and childhood I Antibody response in different types of urinary tract infections caused by coliform bacteria *Br Med J II* 524 1963

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THE CHANGING PANORAMA OF CEREBRAL PALSY IN SWEDEN 1954–1970

I Analysis of the General Changes

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ABSTRACT Hagberg B Hagberg G and Olow I (Department of Paediatrics II Children s Hospital and the Habilitation Unit of Bracke-Östergård Gothenburg Sweden) The changing panorama of cerebral palsy in Sweden 1954–1970 I Analysis of the general changes Acta Paediatr Scand 64 187 1975 —From an unselected series of 560 cases of cerebral palsy in Sweden born 1954–70 the changes in incidence through the four periods 1954–58 1959–62 1963–66 and 1967–70 were analysed The total incidence successively and significantly decreased from 2 2‰ in the first period to 1.3 ‰ in the last This decrease was mainly related to (1) the syndromes of spastic and ataxic diplegia (2) the low birth weight babies and (3) the group with perinatal causes The decrease was not related to any special intelligence quotient or geographical region

KEY WORD Cerebral palsy

Cerebral palsy (CP) comprises a conglomerate of different non progressive motor handicap syndromes grouped together because they involve similar problems of management and similar principles of integration of the various training and learning activities The concept implies however etiologically and pathogenetically very heterogeneous conditions Our knowledge concerning the causes and mechanisms of cerebral palsy has increased considerably during the last decade (8 13 16 17) Recently in a preliminary communication (10) we showed a significantly decreasing incidence of low birth weight diplegias from the mid 1960s in Sweden which was intimately related to changes in the routines for the care of low birth weight babies This indicates that meaningful information concerning the effect of obstetric efforts and preventive and therapeutic measures during the neonatal period can be obtained today Obviously such information may be of importance for our understanding of the pathogenesis of the brain damage It may also have practical implications for obstetric and neonatal care

The present study has been divided into two parts Part I presents the incidence and changes in incidence through the years 1954–70 arrived at from an unselected series of patients deriving from particularly well controlled parts of Sweden In Part II these changes are discussed for each group of syndromes against the background of our data from available records and of our present knowledge of the etiology and pathogenesis

DEFINITIONS AND CLASSIFICATIONS WITH COMMENTS

Cerebral palsy was defined in this study as A disorder of movement and posture due to a defect or le

sion of the immature brain in accordance with traditional criteria (3)

The classification of cerebral palsy syndromes was based on clinical signs—not on possible causes or sites of damage—in accordance with the classification system generally accepted in Sweden since 1958 (1 8 9)

Spastic (hypertonic) syndromes

Among the spastic syndromes *hemiplegias* do not need any definition here The group with *spastic tetraplegia* was restricted in definition to comprise only cases with pareses of the upper limbs of the same degree as or more severe than in the lower limbs The group seems to us to be analogous with the bilateral hemiplegias of Ingram (13) Assigned to the group of *diplegias* were all hypertonic cases where the lower limbs were found to be more affected than the upper ones Thus we included such cases even when they exhibited severe generalized damage where the classification would certainly have been tetraplegia according to some other investigators This differentiation and delineation between diplegias and tetraplegias is important but has often been neglected in earlier series presented in the literature

Ataxic syndromes

The different syndromes within ataxic cerebral palsy are difficult to delimit as has been extensively discussed recently (11 20) For the purpose of the present study we adhered to the subdivision of Ingram (12) into the two main syndromes *congenital ataxia* and *ataxic diplegia* The latter was considered to be present when spasticity of diplegic distribution was found in addition to ataxia

Dyskinetic syndromes

Athetosis was defined as the classical dyskinetic syndrome characterized by incoordinate generalized hyperkinetic movements The movements are choreatic athetoid or a mixture of the two the muscular tone is normal or hypotonic—particularly in early years—and most patients are only moderately disabled Thus we use the term athetosis in a much more restricted way than does Minear for example in his American classification (18) where the term is synonymous with the dyskinetic group in general

To the *dystonic* or *tonus changing* syndromes we assigned the more severely affected dyskinetic forms dominated by dystonic traits tension shifts and persisting neonatal reflex patterns—first described in Scandinavia by Brandt (4) These patients are usually severely disabled in all four limbs as well as in the trunk tongue and pharyngeal musculature Their motor developmental level usually remains at a neonatal or early infantile stage Most of their motor performances are structured from elicitation of various primitive reflexes The majority also have more or less pronounced athetoid hyperkinesias but these do not usually dominate the picture The borderline between the two main syndromes is not sharp and transitional cases exist

Low birth weight was defined as a birth weight ≤2 500 g and was further divided into moderately low birth weight >1 500≤2 500 g and very low birth weight ≤1 500 g

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Perinatal factors were considered to be the cause when obvious abnormalities had occurred just before during or within 7 days after delivery All low birth weight babies normal for date and with a normal pre- and postnatal history were also assigned to this group

Postnatal factors were considered to be the cause in all cases with clear brain-damaging events occurring between the ages of 7 days and 2 years Hydrocephalic patients without obviously abnormal perinatal histories were assumed to have acquired their CP syndrome mainly due to increased brain pressure after the very first months of life (9)

Owing to the varying validity of the information as in all retrospective studies we were forced to make these generalizations in the definitions of causes In the event of more than one possible cause the most probable one was chosen

CLINICAL MATERIAL AND METHODS

All children with any of the syndromes of cerebral palsy and born in 1959-68 in the city of Gothenburg the county of Uppsala and the four counties belonging to the western county region of Sweden were traced They derived from a total population of 1 8 million (1970) They were all re evaluated and classified according to the system and the definitions given above In the few children with more than one CP syndrome the dominant and more severe one was chosen for classification The results of our preliminary study (10) led to an expansion of our series to include children born in 1954-58 and 1969-70 Reliable information from the period 1954-58 could however only be obtained from the city of Gothenburg the number of patients therefore being limited to 65 The total number of patients was finally 560 The series obtained from the various periods were considered to be unselected and complete In contrast to many earlier series all cases of cerebral palsy among the severely mentally retarded in institutions were included The frequency for the low birth weight babies (4 3%) remained unchanged through the years The approximate proportion of deliveries in obstetric units in Sweden during the years in question was 99%

The reliability of the series presented is thought to be

Table 1 *Incidence of CP syndromes during the four periods 1954–58 1959–62 1963–66 1967–70*
Total number of cases 560 Sp/at=spastic/ataxic

		Inc per 1 000 live births				
		54–58	59–62	63–66	67–70	p≤
Spastic syndromes	Hemiplegia	0 79	0 54	0 64	0 55	
	Tetraplegia	0 14	0 08	0 01	0 07	
	Diplegia } Atax diplegia	1 03	0 87	0 59	0 41	0 0005
Ataxic syndromes	Cong ataxia	0 07	0 11	0 14	0 16	
Dyskinetic syndromes		0 21	0 29	0 29	0 15	0 05
Total CP		2 24	1 89	1 67	1 34	0 005
Sp/at diplegic syndromes	B w >2 500 g	0 31	0 33	0 27	0 21	
	B w ≤2 500 g	0 72	0 55	0 32	0 20	0 0005
Number of cases		65	172	182	141	

higher than in most earlier studies owing to the uniformity of the classification and examination Only two (experienced) examiners were involved and the same system of classification with only minor changes had been used since 1958

Within the larger series statistical calculations concerning the differences between the four consecutive periods were performed on each syndrome separately As spastic diplegia and ataxic diplegia partially overlap rendering a strict differentiation almost impossible these two diagnoses were combined for the purpose of this study Conventional statistical methods were used

RESULTS AND DISCUSSION

Total incidence of cerebral palsy

The incidence figures for the total number of cases of cerebral palsy as well as for the different syndromes separately in the four consecutive periods are presented in Table 1 and the changes are illustrated graphically in Fig 1

The total incidence of cerebral palsy decreased successively through the 17 years This decrease is statistically highly significant ($p \leq 0.005$) when comparing the four periods Similar results were recently revealed in a Danish registration series (5) comprising no fewer than 2310 cases and valid for the period 1940–67 the series was particularly complete for the years 1950–65 (6) While the incidence figures for Denmark were found to be about 3 per 1000 in the early fifties they had decreased to about 2 per 1000 in the mid sixties The decrease in this latter series was found to be statistically significant even when particularly tested for the period 1950–65

The figure 2 2 per 1000 live births for the period 1954–58 in the city of Gothenburg seems relatively high compared with other similar series from corresponding periods in Sweden (2 15 19) The present results should however be judged against the fact that this series of cases is highly unselected and includes multi handicapped children i e severely mentally retarded who are usually registered only under this heading and have often not been fully included in earlier retrospective field studies (15)

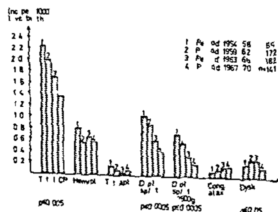


Fig 1 Incidence of CP according to syndrome Sp/at=spastic/ataxic

sion of the immature brain in accordance with traditional criteria (3)

The classification of cerebral palsy syndromes was based on clinical signs—not on possible causes or sites of damage—in accordance with the classification system generally accepted in Sweden since 1958 (1 8 9)

Spastic (hypertonic) syndromes

Among the spastic syndromes *hemiplegias* do not need any definition here The group with *spastic tetraplegia* was restricted in definition to comprise only cases with pareses of the upper limbs of the same degree as or more severe than in the lower limbs The group seems to us to be analogous with the bilateral hemiplegias of Ingram (13) Assigned to the group of *diplegias* were all hypertonic cases where the lower limbs were found to be more affected than the upper ones Thus we included such cases even when they exhibited severe generalized damage where the classification would certainly have been tetraplegia according to some other investigators This differentiation and delineation between diplegias and tetraplegias is important but has often been neglected in earlier series presented in the literature

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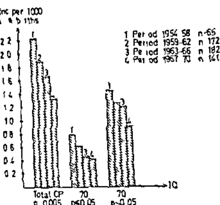


Fig 4 Incidence of CP according to intellectual capacity

the children with a normal and a borderline intelligence quotient ($p \leq 0\ 05$) and among the mentally retarded ones ($p \leq 0\ 05$) Thus it seems that the decrease is not limited to any special IQ group Reservation must be made for the fact that the intelligence grouping was only done roughly

Distribution according to geographical region

An analysis was performed to find out whether there were any differences in total incidence between the city of Gothenburg with its special neonatal units attached to the delivery wards on the one hand and the Uppsala and Western regions without such facilities on the other This comparison was limited to the years 1959–70 No significant differences were found although the city of Gothenburg showed slightly higher figures (1 76% as against 1 57% for the other parts) This might be explained by the significantly higher incidence of children of low birth weight in Gothenburg (4 8%) as compared with the whole of Sweden (4 3%)

GENERAL DISCUSSION

During recent years there has been much debate as to whether the incidence of cerebral palsy has decreased (21) or remained stationary (7) Misgivings have been raised that modern efforts of neonatologists might rescue severely damaged babies—who otherwise would have died—to a miserable life

This study has shown that a significant decrease in cerebral palsy has occurred in Sweden where more than 99% of the deliveries take place in hospital the majority in a special obstetric department with attendant paediatric service The decrease mainly concerns perinatally or neonatally acquired forms of cerebral palsy is particularly valid for low birth weight babies and is not referred to any special intelligence quotient

One possible objection that may be raised against some of our findings is that the youngest of our patients had not reached more than 3 years of age at the end of the study However repeated health surveys of four year olds out of the whole population of Sweden have shown that today new cases of previously hidden cerebral palsy are practically never discovered after the first few years of life (14)

Strong general support for our findings is given by the recent Danish Registration Study (6) From both studies it can be concluded that the very active efforts during recent years to prevent brain damage caused by neonatal jaundice asphyxia and perhaps also severe birth trauma has given more profit in the form of undamaged babies than losses in the form of surviving severely disabled children who would have died with the less active approach of earlier years Where the limits for utmost efforts to resuscitate should be drawn is impossible to state today

REFERENCES

1 d Avignon M Bille B Hagberg B & Olow I Clinical classification of cerebral palsy in childhood *Lakartidn* 57 2533 1960

d Avignon M & Gardenstrom L Cerebral pares hos barn och ungdomar inom Stockholmsområdet En medicinsk och social utredning *Nord Med* 59 55 1958

3 Bax M *Terminology* and classification of cerebral palsy *Dev Med Child Neurol* 6 95 1964

4 Brandt S Paper read at 1 t Nord Conf on cerebral palsy Copenhagen 1954

5 Glenting P Cerebral parese patienter i danska am

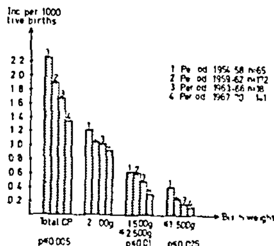


Fig 2 Incidence of CP according to birth weight

Distribution according to syndrome

As seen in Fig 1 the main decrease can be referred to the group spastic/ataxic diplegia and among this group to the low birth weight children ($p \leq 0\ 0005$) In addition a significant decrease ($p \leq 0\ 05$) is noted in the last period among the dyskinetics The other cerebral palsy syndromes do not show any significant changes through the years though the hemiplegias have their highest figures the first period The factors underlying the changes will be further analysed and discussed in Part II of this study

Distribution according to birth weight

When grouped by birth weight the decrease is seen throughout but to a statistically significant degree only among the children with moderately low ($p \leq 0\ 01$) and very low ($p \leq 0\ 025$) birth weight (Fig 2) The distribution of children with low birth weight among the cerebral palsied was 46% in the first period and 32% in the last this latter figure is still extremely high compared with the figures of 4 3% for low birth weight babies among all births in Sweden Thus low birth weight and cerebral palsy are highly correlated to each other

Distribution according to cause

The changes in the four groups of causes are presented in Fig 3 The highest gain is noted in the group with perinatal causes ($p \leq 0\ 0005$) this being quite in line with the birth weight figures as according to our definitions the majority of low birth weight children were referred to this group When all low birth weight children are excluded there is still a slight but not significant decrease in the group with perinatal causes The group with prenatal causes remained statistically unchanged through the years although the figures were highest in the first period All SFD cases were also analysed separately and the figures show that the higher incidence of prenatal causes in the first period is referred to this group The number is too small to allow any further conclusions to be drawn however

The group with an untraceable cause was also unchanged through the years which in our opinion might indicate a prenatal rather than a perinatal cause in these cases

To conclude it may be said that the proportion of cerebral palsy due to perinatal factors decreased from over 50% in the first period to 40% in the last one Further achievements might be expected in this group as a result of advances in obstetric and neonatal care and technique

Distribution according to intellectual capacity

Very gratifying we found (Fig 4) that a successive decrease took place both among

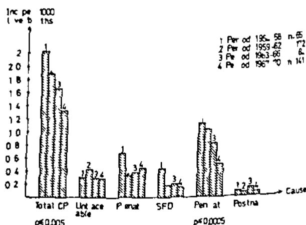


Fig 3 Incidence of CP according to cause

Acta Pædiatr Scand 64 193–200 1975

THE CHANGING PANORAMA OF CEREBRAL PALSY IN SWEDEN 1954–1970

II *Analysis of the Various Syndromes*

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From the Department of Paediatrics II Children s Hospital and the Habilitation Unit of Bracke Östergard Gothenburg Sweden

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KEY WORD Cerebral palsy

In part I of this investigation the general numerical changes of the cerebral palsy syndromes through the years 1954 to 1970 were investigated from an unselected Swedish series of 560 cases This second part is an analysis of data obtained from each main syndrome separately undertaken with the aim of obtaining more detailed information concerning clustering of factors of known or suspected relevance for the etiology pathogenesis and changes in incidence

CLINICAL MATERIAL AND METHODS

Data concerning the mode of sampling of the clinical series and also the definitions criteria and classifications used for dividing the series into 8 main syndromes have all been given in part I For analysis of changes through the years the clinical material was divided into four periods according to the birth years of the children 1 (1954–58) (1959–6.) 3 (1963–66) and 4 (1967–70)

RESULTS AND DISCUSSION

Spastic Syndromes (*n*=405)

The group as a whole comprised a large number of etiologically and pathogenetically very different conditions It was therefore considered purposeless to analyse the figures for the total group through the four periods

Spastic hemiplegia (*n*=200 Tables 1–4)

Incidence No significant changes in incidence took place within our group of spastic hemiplegia through the periods 1–4 though the highest figures were obtained in period 1 It is of interest to note that in a large Danish registration series of 440 hemiplegias from the years 1950–69 Glenting (5) demonstrated a successive significant decrease after 1960

ter øst for Storebælt født i årene 1940–1969 *Meddelelser fra CP registret I* 1973
6 Glenting P Personal communication 1973
7 Griffiths M I Cerebral palsy in Birmingham *Dev Med Child Neurol 9* 33 1967
8 Gustavson K H Hagberg B & Sanner G Identical syndromes of cerebral palsy in the same family *Acta Paediatr Scand 58* 330 1969
9 Hagberg B & Naglo A S The conservative management of infantile hydrocephalus *Acta Paediatr Scand 61* 165 1972
10 Hagberg B Olow I & Hagberg G Decreasing incidence of low birth weight diplegia—an achievement of modern neonatal care? *Acta Paediatr Scand* 62 199 1973
11 Hagberg B Sanner G & Steen M The dysequilibrium syndrome in cerebral palsy *Acta Paediatr Scand* Suppl 226 1972
12 Ingram T T S Congenital ataxic syndromes in cerebral palsy *Acta Paediatr Scand 51* 209 1962
13 — *Paediatric aspects of cerebral palsy* E & F Livingstone Ltd Edinburgh and London 1964
14 Köhler L Physical examination of four year-old children *Acta Paediatr Scand 62* 181 1973
15 Lagergren J Motor handicapped children A study from a Swedish country *Dev Med Child Neurol 12* 56 1970
16 Masland R L Spastic diplegia after short gestation *Dev Med Child Neurology 12* 127 1970
17 McDonald A Children of very low birth weight *MEIU Res Monogr 1* 1967
18 Minear W L A classification of cerebral palsy *Pediatrics 18* 841 1956
19 Olow I & Skottsberg A M Behovet av specialskola för barn med cerebral pares *Social med Tidskrift 35* 108 1958
20 Sanner G & Hagberg B 188 cases of nonprogressive ataxic syndromes in childhood Aspects of aetiology and classification *Neuropadiatrie 5* 2 4 1974
21 Woods G L A lowered incidence of infantile cerebral palsy *Dev Med Child Neurol 5* 449 1963

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Table 1 *Incidence of the different CP syndromes during the four periods 1954–58 1959–62 1963–66 1967–70*

		54–58 (‰)	59–62 (‰)	63–66 (‰)	67–70 (‰)
Hemiplegia	*n*=200	0.79	0.54	0.64	0.55
Tetraplegia	*n*=19	0.14	0.08	0.01	0.07
Sp diplegia	*n*=186	0.96	0.71	0.51	0.35
At diplegia	*n*=31	0.07	0.16	0.08	0.06
Cong ataxia	*n*=44	0.07	0.11	0.14	0.16
Athetosis	*n*=20	0.04	0.10	0.05	0.04
Dystonic forms	*n*=60	0.17	0.19	0.24	0.11
Total CP	*n*=560	2.24	1.82	1.67	1.34

Birth weight The number of low birth weight children was 59/200 (30%) the distribution in the four periods varying between 35 and 26% 20/200 (10%) were small for date (SFD) no fewer than 6 of them deriving from the small series of the first period comprising only 23 hemiplegias However the small number of SFD cases allowed no definite conclusions to be drawn

Cause The four groups of causes did not change through the periods either in incidence or in distribution In as many as 61/200 (30%) of the hemiplegias in our series the cause was untraceable a figure which was significantly higher ($p \leq 0.002$) than the mean for the total series of cerebral palsy i e 115/560 (21%) A larger proportion of untraceable causes (41%) was only found among congenital ataxias In our opinion the constancy of the incidence of untraceable causes through the periods and its similarity to the distribution in congenital ataxia suggests that these cases are of prenatal origin Hemiplegia due to obvious prenatal factors was found in 45/200 (23%) of the cases In 39/45 (87%) the prenatal grouping was based on fetal undernutrition (SFD and/or infarction of the placenta and/or repeated bleedings during pregnancy) The surprisingly high proportion of prenatal cases in our series agrees closely with French observations (6 15)

In 82/200 (41%) perinatal factors were considered of pathogenetic relevance and there were as many babies with a normal as with a low birth weight in this group Moderate to severe asphyxia had occurred in practically all these cases this was combined with signs of intracranial haemorrhage in 11 cases and with icterus motivating blood exchange transfusions in 13 cases Significantly ($p \leq 0.005$) more blood exchange transfusions had been performed among the hemiplegias (8%) than among all the other CP syndromes together (3%) Further analysis is required to explain this observation In 12/200 (6%) the cause was considered to be postnatal infections being predominant as in 8/12 of our patients Of the remaining 3 had had severe hypoxic conditions and one a skull injury

IQ Brain damaging mechanisms causing spastic hemiplegia seemed to affect mental development less than those producing other CP syndromes except for athetosis In our

Table 2 *The distribution of birth weight in the CP syndromes*

		≤1 500 g (%)	>1 500 ≤2 500 g (%)	>2 500 g (%)	SFD (%)
Hemiplegia	*n*=200	4	25	71	10
Tetraplegia	*n*=19	5	27	68	26
Sp diplegia	*n*=186	22	39	39	16
At diplegia	*n*=31	13	32	55	19
Cong ataxia	*n*=44	0	4	96	0
Athetosis	*n*=20	10	15	75	5
Dystonic forms	*n*=60	15	22	63	15
Total CP	*n*=560	12	28	60	12

Table 3 *The distribution of causes in the different CP syndromes*

		Untraceable (%)	Prenatal (%)	Perinatal (%)	Postnatal (%)
Hemiplegia	$n=200$	30	23	41	6
Tetraplegia	$n=19$	10	42	37	11
Sp diplegia	$n=186$	15	25	57	3
At diplegia	$n=31$	13	45	39	3
Cong ataxia	$n=44$	41	25	18	16
Athetosis	$n=20$	10	5	85	0
Dystonic forms	$n=60$	2	26	65	7
Total CP	$n=560$	21	25	48	6

series 85% of all hemiplegias were intellectually normal or borderline (IQ≥70)

Spastic tetraplegia ($n=19$ Tables 1–4)

Incidence The syndrome of spastic tetraplegia when restricted in definition to comprise only cases where the spastic pareses of the upper limbs was of the same degree as or more severe than that of the lower limbs was found in only a small number of patients which remained relatively constant through the four periods

Birth weight Six of these 19 patients (32%) had birth weights less than 2 500 g and of these no fewer than 5 were SFD

Cause In 2 cases no obvious causative factors were found Prenatal factors were found to predominate (42%) and among this group toxicosis in the mother and/or SFD were the most common findings All cases in the perinatal group (37%) had severe asphyxia as one component Two patients had an abnormal postnatal history one of them had had a severe infection and the other had had complications from pneumoencephalography Altogether 15/19 cases had shown evidence of intra uterine perinatal or post natal asphyxia

IQ Concerning intelligence this was the very worst group all of them being severely mentally retarded

This group seems to be analogous on the whole with Ingram s bilateral hemiplegias (14) As in Ingram s series our patients were all severely disabled and multi handicapped with microcephaly severe mental retardation epilepsy and pseudobulbar pareses

Spastic diplegia ($n=186$ Tables 1–4)

Incidence The most intriguing incidence changes through the four periods were noted for the diplegic children Statistically a highly significant ($p \leq 0\ 0005$) successive decrease was revealed In fact the diminishing incidence among the spastic and ataxic diplegics accounted for practically the entire decrease in the total CP incidence (Fig 1)

Birth weight The decrease was specifically and significantly limited to cases with a birth weight less than 2500 g (Fig 1) The decline since the mid sixties mainly concerned babies with a birth weight ≤2000 g (Table 5) This trend was recently confirmed in England by the Hammersmith group (4) who for the period 1961–64 noted 6 diplegics

Table 4 *The distribution of intellectual capacity in the different CP syndromes*

		IQ <70 (%)	IQ ≥70 (%)
Hemiplegia	$n=200$	15	85
Tetraplegia	$n=19$	100	0
Sp diplegia	$n=186$	31	69
At diplegia	$n=31$	29	71
Cong ataxia	$n=44$	48	52
Athetosis	$n=20$	5	95
Dystonic forms	$n=60$	62	38
Total CP	$n=560$	31	69

Table 1 *Incidence of the different CP syndromes during the four periods 1954–58 1959–62 1963–66 1967–70*

		54–58 (‰)	59–62 (‰)	63–66 (‰)	67–70 (‰)
Hemiplegia	$n=200$	0.79	0.54	0.64	0.55
Tetraplegia	$n=19$	0.14	0.08	0.01	0.07
Sp diplegia	$n=186$	0.96	0.71	0.51	0.35
At diplegia	$n=31$	0.07	0.16	0.08	0.06
Cong ataxia	$n=44$	0.07	0.11	0.14	0.16
Athetosis	$n=20$	0.04	0.10	0.05	0.04
Dystonic forms	$n=60$	0.17	0.19	0.24	0.11
Total CP	$n=560$	2.24	1.89	1.67	1.34

Birth weight The number of low birth weight children was 59/200 (30%) the distribution in the four periods varying between 35 and 26% 20/200 (10%) were small for date (SFD) no fewer than 6 of them deriving from the small series of the first period comprising only 23 hemiplegias However the small number of SFD cases allowed no definite conclusions to be drawn

Cause The four groups of causes did not change through the periods either in incidence or in distribution In as many as 61/200 (30%) of the hemiplegias in our series the cause was untraceable a figure which was significantly higher ($p<0.002$) than the mean for the total series of cerebral palsy i e 115/560 (21%) A larger proportion of untraceable causes (41%) was only found among congenital ataxias In our opinion the constancy of the incidence of untraceable causes through the periods and its similarity to the distribution in congenital ataxia suggests that these cases are of prenatal origin Hemiplegia due to obvious prenatal factors was found in 45/200 (23%) of the cases In 39/45 (87%) the prenatal grouping was based on fetal undernutrition (SFD and/or infarction of the placenta and/or repeated bleedings during pregnancy) The surprisingly high proportion of prenatal cases in our series agrees closely with French observations (6 15)

In 82/200 (41%) perinatal factors were considered of pathogenetic relevance and there were as many babies with a normal as with a low birth weight in this group Moderate to severe asphyxia had occurred in practically all these cases this was combined with signs of intracranial haemorrhage in 11 cases and with icterus motivating blood exchange transfusions in 13 cases Significantly ($p\leq0.005$) more blood exchange transfusions had been performed among the hemiplegias (8%) than among all the other CP syndromes together (3%) Further analysis is required to explain this observation In 12/200 (6%) the cause was considered to be postnatal infections being predominant as in 8/12 of our patients Of the remaining 3 had had severe hypoxic conditions and one a skull injury

IQ Brain damaging mechanisms causing spastic hemiplegia seemed to affect mental development less than those producing other CP syndromes except for athetosis In our

Table 2 *The distribution of birth weight in the CP syndromes*

		≤1 500 g (%)	>1 500 ≤2 500 g (%)	>2 500 g (%)	SFD (%)
Hemiplegia	$n=200$	4	25	71	10
Tetraplegia	$n=19$	5	27	68	26
Sp diplegia	$n=186$	22	39	39	16
At diplegia	$n=31$	13	32	55	19
Cong ataxia	$n=44$	0	4	96	0
Athetosis	$n=20$	10	15	75	5
Dystonic forms	$n=60$	15	22	63	15
Total CP	$n=560$	12	28	60	12

ot justified Ataxia within the concept of cerebral palsy constitutes a rag bag for several less well delimited syndromes (11 20) Classification into the two main sub groups congenital ataxia and ataxic diplegia 13) has however been found profitable and has been widely accepted In our series these two sub groups partly contain etiologically very differently composed syndromes as exemplified by the fact that children with a low birth weight constituted nearly half of the patients in our series of ataxic diplegia while a low birth weight was very rarely encountered in congenital ataxia In fact the majority of the present series of ataxic diplegic children revealed more clinical and pathogenetic similarities to spastic diplegia and these two groups were therefore combined for statistical analysis The borderline between them was extremely difficult to demarcate and varied even between two well synchronized examiners

Ataxic diplegia (n=31 Tables 1–4)

Incidence After 1959 there was a successive decline in the incidence of this group which was grossly parallel to that of spastic diplegia Thus there were three *times* as many cases in 1959–62 as in 1967–70 However the number of cases in this series is too small to allow any statistically valid conclusions to be drawn for the group separately

Birth weight Among the 31 cases 14 (45%) had a birth weight less than 2500 g Only one of these belonged to the last period 6/31 (19%) were SFD

Cause In 4/31 (13%) the origin was untraceable but might have been due to genetic disorders Genetic factors have been found to constitute one important group in full term non hydrocephalic ataxic *diplegic infants* (7 20) Prenatal causes were probable in 14/31 (45%) cases 8 of them being related to SFD and/or bleeding in the late stage of pregnancy In this series perinatal factors characterized 12/31 (39%) of the cases in 9 of these there was a history of perinatal asphyxia sometimes in combination with icterus Two were premature babies without any obvious perinatal or neonatal problems Ataxic diplegia is also known to be the characteristic postnatally acquired cerebral palsy syndrome of infantile hydrocephalus (12 20) The one child with postnatal damage belonged to this group

IQ With regard to intelligence these children were similar to the corresponding simple spastic diplegics (71% normal or borderline)

It is evident from the above data that our group of ataxic diplegic children mainly had intimate etiological connections with spastic diplegia and only to a minor extent with congenital ataxia For future purposes it would seem more correct therefore to classify ataxic diplegic patients as a sub group preferably related to those with pure spastic diplegia

Congenital ataxia (n=44 Table 1–4)

Incidence The number of children with congenital ataxia remained unchanged through the years under study The *non significantly* lower figures for the first period are either accidental or—more probably—due to a less refined examination technique *for revealing less* obvious ataxic syndromes *at that time particularly* as regards ataxia in mentally subnormal children

Birth weight Congenital ataxia was the only CP syndrome in which the birth weight distribution was the same as for the total population In our series 96% were full term babies and only 4% had low birth weights which is highly discrepant from all the other CP syndromes No small for date children were found among the patients with congenital ataxia

Cause In 18/44 (41%) of the cases *no* causes were traced and in 11/44 (25%) prenatal factors in the histories could have been responsible In Sweden it has been found in recent years that hereditary forms of cerebral palsy are especially numerous within the syndrome of congenital ataxia partic

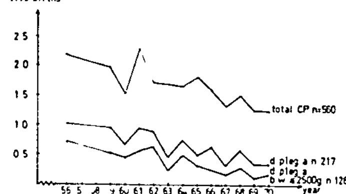


Fig 1 Incidence of spastic/ataxic diplegia through the years 1954–70 compared with the total incidence of CP

among 58 babies with a birth weight less than 1 500 g while the corresponding figures for the period 1965–70 were 0 among 107 with very low birth weights

Spastic diplegia is said to be the disease of the child born immature (16) Although the proportion of spastic dipareses in children born prematurely did decrease from 68% in period 1 to 54% in period 4 this statement still holds good for babies born up to 1970

Cause In 28/186 (15%) of the cases the cause was untraceable Prenatal causes were probable in 46/186 (25%) and in 33 of these (72%) this grouping was based on fetal undernutrition (SFD and/or infarction of the placenta and/or repeated bleedings during pregnancy) Parallel to the decrease among the spastic diplegics with a low birth weight we found a significant ($p \leq 0\ 0005$) successive decrease in the incidence of cases with perinatal causes from 0 66‰ in period 1 to 0 13‰ in period 4 There was a history of asphyxia with or without simultaneous icterus in 60/107 (56%) of the cases with a perinatal origin but only in a few of them were these conditions severe 44/107 (41%) were low birth weight babies with no obvious complications All but one of the postnatal causative factors (5/186 cases 3%) consisted of hydrocephalic states

IQ 129/186 (69%) of the spastic diplegic children had a normal or borderline intelligence

Table 5 *Number of low birth weight diplegics*

	b 1959–64	b 1965–70
>2 000≤2 500 g	18	17
>1 500≤2 000 g	29	13
≤1 500 g	23	17
All diplegics	112	75
All with a low birth weight	6 210	6 965

In a preliminary study covering only the years 1959–68 (10) a decreasing incidence of dipareses was noted in children with a low birth weight from the middle of the 1960s This coincided in time with the introduction of new routine procedures in the care of low birth weight babies in Sweden In particular the active effort against acidosis hypocaloric states and hypoglycemia *ad modum* Usher (21) were believed to be of importance The extended study including in addition period 1 (1954–58) does not contradict this assumption However our present figures indicate that the decrease had already started at least 5 years earlier demonstrating that the improvement for low birth weight babies can not be attributed exclusively to the Usher regimen

Spastic diplegia in infants born at term is usually not associated with a history of perinatal complications (14) A clustering of abnormalities in the mothers during their pregnancies and in the siblings of the patients was revealed by Ingram (14) supporting the view that in a large proportion of the children with this form of brain damage the causative factors are prenatal The unchanged incidence in our series of diplegic children born at term including small for date babies is in good agreement with this view as most of the prenatal factors cannot as yet be influenced

Ataxic Forms

A statistical calculation on the changes in incidence of the ataxic forms as an entity is

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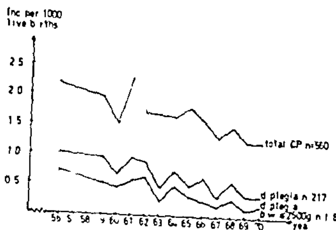


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tonic patient in Sweden is a slightly handicapped child with a normal intelligence born at term and with a history suggesting perinatal asphyxia sometimes combined with moderately raised serum bilirubin

Dystonic forms (*tension athetosis*)
(n=60 Tables 1–4)

These syndromes are often difficult to trace in the literature They are not seldom wrongly hidden within the group of spastic tetraplegia or more logically placed as sub groups within athetoid cerebral palsy as in Minear s classification in the US (17) In Scandinavia they were first delineated by Brandt (2) and called tetraplegia hypersynkinetica or dystonica For practical reasons we have coined the designation *tonus changing syndrome* for this group in Sweden from one of the dominant signs the often extremely variable muscular tonus manifested in rapid tension shifts

Incidence In our previous study reported in 1960 (1) this group constituted not less than 25% of that series (628 cases) In the present series the corresponding figure is 10% It is therefore tempting to believe that these forms of cerebral palsy have decreased as compared with the situation in the 1940s and early 1950s However our series presented in 1960 was not unselected severe cases constituting the majority of the patients admitted at that time to our few CP centres No unselected series from these early periods are available When our present group was analysed separately after 1954 it showed no significant differences in incidence though there was a decrease in period 4 However it is important to stress that modern methods for resuscitation of asphyxiated neonates have not brought about an increasing number of severely handicapped survivors

Birth weight 22/60 (37%) were children with a low birth weight and no fewer than 10/60 (15%) were SFD The decrease in period 4 was not related to any special birth weight

Cause In only 1/60 (2%) could no cause be traced Prenatal factors were noted in 16/60 (26%) in 10 of them these comprised infarction of the placenta and/or small for date and/or toxicosis in the mother A special section among the dystonics comprises certain children with primary forms of microencephaly and an arrested mental and motor development at an extremely primitive level (1) Only 2 children of this type were found in the present series In 39 of the 60 cases (65%) there was a history of injurious perinatal factors the majority (32/39 82%) had had perinatal asphyxia in a large percentage evaluated as severe Only 3 cases due to kernicterus were noted one in the city of Gothenburg in period 1 and two in the Western region in period 2 and deriving from small distant delivery units Postnatal causes were probable in 4/60 (7%) of the cases Two of them were considered to be due to severe asphyxia and the other two to infectious or parainfectious processes

IQ The most tragic situations within cerebral palsy are to be found among the dystonic patients not few of them (in our series 38%) being intellectually normal or only slightly subnormal but at the same time lacking any purposeful motor function and with a pattern of movements on a primitive neonatal level

REFERENCES

1 d Avignon M Bille B Hagberg B & Olow I Clinical classification of cerebral palsy in childhood *Lakartidn 57* 2533 1960
2 Brandt S Paper read a 1st *Nord Conf* on Cerebral Palsy Copenhagen 1954
3 Crothers B & Paine R S *The natural history of cereb al palsy* Oxford University Press 1959
4 Davies P Personal communication 1973
5 Clenting P Cerebral parese patienter i dansk amter ost for Storebelt født i årene 1940–1969 *Meddelelser fra CP registret 1* 1973
6 Goutierec F Challamel M J Aicardi J & Gilly R Les hémiplégies congénitales *Arch Fr Pédiatr 29* 839 1972

ularly when combined with mental retardation (7) For the dysequilibrium syndrome an autosomal recessive inheritance is the rule (11 20) No genealogic investigations were performed in this series but on the basis of the above observation we suppose the cases with untraceable causes to have a prenatal mainly genetic background In 8 of the 44 cases (18%) the cause was assumed to be perinatal and in 7 of them this was based on a history of asphyxia Postnatally acquired forms are usually related to para/postinfectious or post traumatic factors or are secondary to hydrocephalic states (20) This was also the case in our series where out of 7 children (16%) with a postnatal cause 3 were considered to be due to postinfectious 1 to post traumatic and 3 to hydrocephalic states The first neurologic signs in slight to moderate hydrocephalic states are gait retardation hypotonia in the lower limbs and dyssynergia (8 12)

IQ Just as many children (52%) were of normal or borderline intelligence as were mentally retarded

In the light of the marked predominance of untraceable and prenatal factors and with the age criteria for cerebral palsy in our studies in mind major changes in the incidence of cong ataxia through the years would have been surprising The decrease which was also found in this group in the Danish series presented by Glenting (5) might be explained by the fact that he also included children in whom the ataxic syndrome had been acquired in later childhood whereas our limit was put at ≤2 years of age for postnatal cases

Dyskinetic Syndromes ($n=80$)

This group as a whole is etiologically and pathogenetically less heterogeneous than the group of spastic syndromes and it therefore seemed of interest to analyse its total figures In fact many earlier investigators have treated all these patients under one heading —dyskinesia (3 14) or athetosis (17 18) A significantly decreasing incidence of the dyskinetic syndromes was apparent during period 4 ($p \leq 0.05$) i e not until the end of the 1960s The two main causes are known to be kernicterus and perinatal asphyxia The major changes within the group and related to the icteric children obviously had occurred in Sweden before 1954 (1) as a result of a regional system for prophylactic exchange transfusions which was initiated in the early 1950s The trend from 1954 onwards thus mainly parallels the effects of asphyxia occurring late in pregnancy during labour or at delivery In only 3/80 (4%) could no cause be traced This figure is significantly less ($p \leq 0.0001$) than in the other CP syndromes

Athetosis (non tension athetosis)
($n=20$ Tables 1–4)

The total number of athetotic patients is too small to allow any statistical conclusions to be drawn

Birth weight 5/20 (25%) were children with low birth weight

Cause Previously the main cause of athetosis was kernicterus (19) In the present series this only applied to one case in the city of Gothenburg in 1954 and in two cases in the western region in period 2 14/20 (70%) had perinatal asphyxia as the probable cause 4 of them in combination with slight to moderate icterus One had a genetically determined form a simple dominant inheritance being probable (9) In the remaining two cases no cause could be traced

IQ This group was outstanding as regards intelligence as 19/20 (95%) had a normal or borderline capacity (IQ≥70)

To conclude it may be said that icterus as the sole or main cause of a choreoathetotic syndrome has been eradicated in Sweden for more than 15 years It may remain as an additional factor in occasional moderately asphyxiated neonates Nowadays the typical athe

otic patient in Sweden is a slightly handicapped child with a normal intelligence born at term and with a history suggesting perinatal asphyxia sometimes combined with moderately raised serum bilirubin

Dystonic forms (tension athetosis) (*n*=60 Tables 1–4)

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Birth weight 22/60 (37%) were children with a low birth weight and no fewer than 10/60 (15%) were SFD The decrease in period 4 was not related to any special birth weight

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REFERENCES

1 d Avignon M Bille B Hagberg B & Olow I Clinical classification of cerebral palsy in childhood *Lakartidn* 57 2533 1960
2 Brandt S Paper read a 1st Nord Conf on Cerebral Palsy Copenhagen 1954
3 Crothers B & Paine R S *The natural history of cerebral palsy* Oxford University Press 1959
4 Davies P Personal communication 1973
5 Glenting P Cerebral parese patienter i dansk amter øst for Storebelt født i årene 1940–1969 *Meddelelser fra CP registret I* 1973
6 Goutieres F Challamel M J Aicardi J & Gilly R Les hémiplégies congénitales *Arch Fr Pediatr* 29 839 1972

7 Gustavson K H Hagberg B & Sanner G Identical syndromes of cerebral palsy in the same family *Acta Paediatr Scand 58* 330 1969
8 Hagberg B The sequelae of spontaneously arrested infantile hydrocephalus *Dev Med Child Neurol 4* 583 1962
9 Hagberg B Unpublished observations 1974
10 Hagberg B Olow I & Hagberg G Decreasing incidence of low birth weight diplegia—an achievement of modern neonatal care? *Acta Paediatr 62* 199 1973
11 Hagberg B Sanner G & Steen M The dysequilibrium syndrome in cerebral palsy *Acta Paediatr Scand* Suppl *226* 1972
12 Hagberg B & Sjögren I The chronic brain syndrome of infantile hydrocephalus *Am J Dis Child 112* 189 1966
13 Ingram T T S Congenital ataxic syndromes in cerebral palsy *Acta Paediatr Scand 51* 209 1962
14 Ingram T T S *Paediatric aspects of cerebral palsy* E & F Livingstone Ltd Edinburgh and London 1964
15 Lyon G *Contribution a l étude des hémiplegies survenant au cours des premiers mois de la vie* Dactylo Sorbonne Paris 1956
16 Masland R L Spastic diplegia after short gestation *Dev Med Child Neurol 12* 177 1970
17 Minear W L A classification of cerebral palsy *Pediatrics 18* 841 1954
18 Phelps W M Etiology and diagnostic classification of cerebral palsy *Nerv Child 7* 10 1950
19 Plum P Etiology of congenital athetosis *Ann Paediatr Fenn 3* 589 1957
20 Sanner G & Hagberg B 188 cases of non progressive ataxic syndromes in childhood Aspects of aetiology and classification *Neuropadiatrie 5* 224 1974
21 Usher R Reduction of mortality from respiratory distress syndrome of prematurity with early administration of intravenous glucose and sodium bicarbonate *Pediatrics 32* 966 1963

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LEVEL DIAGNOSIS OF SYMPTOMATIC URINARY TRACT INFECTIONS IN CHILDHOOD

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ABSTRACT Jodal U Lindberg U and Lincoln K (Department of Paediatrics and Institute of Medical Microbiology University of Goteborg Goteborg Sweden) Level diagnosis of symptomatic urinary tract infections in childhood Acta Paediatr Scand 64 201 1975.—As no method for localization of urinary tract infection has been shown to be absolutely reliable six procedures have been carried out simultaneously in 25 girls with acute symptomatic infections and the reliability of each method assessed While clinical diagnosis of pyelonephritis or cystitis correlated well with the overall results of the battery of tests the reliability of individual tests varied Highest reliability was obtained with CRP determinations followed by antibody titration sedimentation rate and renal concentrating capacity In the bladder washout test only 8 of the 14 patients with pyelonephritis had findings clearly indicating high infection Intermittent or inadequate discharge of bacteria from the renal parenchyma is suggested as the major source for this inaccuracy In fact half of the 42 final washout specimens from girls with acute pyelonephritis contained less than 1 000 bacteria per ml indicating that low numbers of organisms in ureteric urine is common in childhood pyelonephritis

KEY WORDS Urinary tract infection level diagnosis *E coli* immunology C reactive protein bladder washout kidney concentrating capacity children

Kidney scarring and progressive renal damage seems to develop in some patients with urinary tract infection (UTI) (2 10 13 15 28) Since patients with bacteriuria of renal origin are probably those at risk it may be of importance to *define the level of involvement in UTI* Symptoms like high fever loin and back pain signs such as kidney tenderness and laboratory findings of increased sedimentation rate *transiently* decreased renal concentrating capacity and elevated antibacterial antibody titres in the serum have been used to provide evidence of renal infection (16 19 22 23 27) Bacteriologic evidence for the site of UTI may be derived from ureteric catheterization after primary bladder irrigation (19 21 25) or from a simpler bladder washout technique (4) While the bacteriologic methods are not as readily applicable in clinical practice they may if reliable provide a basis for the evaluation of more easily derived *information* To our knowledge however no critical *evaluation* of the reliability of the bladder washout test has been performed

In a recent study of adults with UTI Fairley et al (4) used the washout test as the main criterion for the level of infection This study indicated a poor correlation between washout results symptoms and serum antibodies to the urinary isolate These findings are in contrast to investigations in children with UTI which have shown correlation between elevated antibody titres and renal involvement as indicated by clinical signs and measurements of con

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LEVEL DIAGNOSIS OF SYMPTOMATIC URINARY TRACT INFECTIONS IN CHILDHOOD

U JODAL U LINDBERG and K LINCOLN

From the Department of Pediatrics and Institute of Medical Microbiology University of Coteborg Goteborg Sweden

ABSTRACT Jodal U Lindberg U and Lincoln K (Department of Paediatrics and Institute of Medical Microbiology University of Goteborg Goteborg Sweden) Level diagnosis of symptomatic urinary tract infections in childhood Acta Paediatr Scand 64 201 1975.—As no method for localization of urinary tract infection has been shown to be absolutely reliable six procedures have been carried out simultaneously in 25 girls with acute symptomatic infections and the reliability of each method assessed While clinical diagnosis of pyelonephritis or cystitis correlated well with the overall results of the battery of tests the reliability of individual tests varied Highest reliability was obtained with CRP determinations followed by antibody titration sedimentation rate and renal concentrating capacity In the bladder washout test only 8 of the 14 patients with pyelonephritis had findings clearly indicating high infection Intermittent or inadequate discharge of bacteria from the renal parenchyma is suggested as the major source for this inaccuracy In fact half of the 42 final washout specimens from girls with acute pyelonephritis contained less than 1 000 bacteria per *ml* Indicating that low numbers of organisms in ureteric urine is common in childhood pyelonephritis

KEY WORDS Urinary tract infection level diagnosis *E coli immunology* C reactive protein bladder washout, kidney concentrating capacity children

Kidney scarring and progressive renal damage seems to develop in some patients with urinary tract infection (UTI) (2 10 13 15 28) Since patients with bacteriuria of renal origin are probably those at risk it may be of importance to define the level of involvement in UTI Symptoms like high fever loin and back pain signs such as kidney tenderness and laboratory findings of increased sedimentation rate transiently decreased renal concentrating capacity and elevated antibacterial antibody titres in the serum have been used to provide evidence of renal infection (16 19 22 23 27) Bacteriologic evidence for the site of UTI may be derived from ureteric catheterization after primary bladder irrigation (19 21 25) or from a simpler bladder washout technique (4) While the bacteriologic methods are not as readily applicable in clinical practice they may if reliable provide a basis for the evaluation of more easily derived information To our knowledge however no critical evaluation of the reliability of the bladder washout test has been performed

In a recent study of adults with UTI Fairley et al (4) used the washout test as the main criterion for the level of infection This study indicated a poor correlation between washout results symptoms and serum antibodies to the urinary isolate These findings are in contrast to investigations in children with UTI which have shown correlation between elevated antibody titres and renal involvement as indicated by clinical signs and measurements of con

7 Gustavson K H Hagberg B & Sanner G Identical syndromes of cerebral palsy in the same family *Acta Paediatr Scand 58* 330 1969
8 Hagberg B The sequelae of spontaneously arrested infantile hydrocephalus *Dev Med Child Neurol 4* 583 1962
9 Hagberg B Unpublished observations 1974
10 Hagberg B Olow I & Hagberg G Decreasing incidence of low birth weight diplegia—an achievement of modern neonatal care? *Acta Paediatr 6?* 199 1973
11 Hagberg B Sanner G & Steen M The dysequilibrium syndrome in cerebral palsy *Acta Paediatr Scand* Suppl 226 1972
12 Hagberg B & Sjögren I The chronic brain syndrome of infantile hydrocephalus *Am J Dis Child 11?* 189 1966
13 Ingram T T S Congenital ataxic syndromes in cerebral palsy *Acta Paediatr Scand 51* 20? 196?
14 Ingram T T S *Paediatric aspects of cerebral palsy* E & S Livingstone Ltd Edinburgh and London 1964
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Table 1. *Clinical and laboratory findings for each patient*

ND=not done. E=E. coli. O=O group. N=non-typable. R=rough (spontaneously agglutinating). ec=enterococci. r( )=reflux (grade). p.r.=parenchymal reduction.

Pat no	Age yrs	Previous known UTI	Temp (°C)	CRP (μg/ml)	Sed rate (mm/h)	Conc capacity (mOsm/l)	Type of bacteria	Antibody titre reciprocal Serum Unreduced	Reduced
1	5	0	40.0	74	43	612	E O 2	8 000	16
2	6	0	40.1	58	20	938	ec	ND	ND
3	8	6	40.2	32	12	807	ec	ND	ND
4	9	6	40.1	25	14	924	E O N	256	16
5	9	1	40.1	300	35	467	E O 25	2 000	512
6	11	>10	39.5	61	28	944	E O N	64	4
7	11	1	40.3	70	25	675	E O 7	1 000	128
8	17	1	39.9	55	62	670	E O N	512	256
9	8	2	39.2	28	25	500	E O 7	2 000	128
10	10	2	39.9	260	32	932	E O N	8 000	16
11	12	2	39.7	29	36	597	E O 1	256	128
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14	12	1	39.8	68	33	635	E O 2	512	512
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17	6	1	37.8	Neg	13	797	E O 4	4	0
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19	7	2	37.0	Neg	10	1 044	E O 2	8	[illegible]
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21	10	0	37.6	Neg	13	930	E O 25	2	0
22	12	1	36.8	Neg	4	960	E O 85	32	8
23	16	0	37.3	Neg	13	993	E O 3	32	0
24	7	1	37.2	Neg	15	925	E O 7	256	16
25	12	7	37.7	Neg	18	963	E O R	128	4

centrating capacity (8, 27) or by ureteric catheterization (9). These divergent findings might be due to differences in the patient materials such as age, sex and number of previous infections or by differences in diagnostic criteria employed. The timing of the serum sampling in relation to the onset of the infection is also important (11, 23) and measurement of antibodies in unreduced (mainly IgM) and reduced (mainly IgG) sera might be of further diagnostic value (8, 24). In the present investigation we have compared different methods for determining the level diagnosis of UTI including bladder washout and serum antibody titration. The material was strictly defined, consisting only of girls with an acute attack of symptomatic UTI.

MATERIAL AND METHODS

The patients were 25 girls age 5–17 years presenting at the emergency room of the Children's Hospital, Göteborg, with acute symptoms. They represent a consecutive series of girls with acute urinary tract infection in whom bladder washout could be performed. Since the cooperation of the children is essential in this test girls under 5 years of age have not been investigated.

According to symptoms and signs a clinical diagnosis of pyelonephritis or cystitis was made. Thus 14 patients (nos. 1–14) were classified as having acute *pyelonephritis*, all with high fever (>39°C) together with loin or high abdominal pain. Twelve of these showed renal tenderness but only three had micturition symptoms. A diagnosis of acute cystitis was made in 10 patients (*nos.* 16–25) with burning and frequency but temperature not exceeding 38°C, none of these had high abdominal or loin pain. In one patient (no. 15) a clinical level diagnosis could not be made. She presented with acute micturition symptoms, temperature of 37.3°C but only slight pain and tenderness of the left loin.

In urine obtained as a midstream specimen all the

ladder washout test acterial *count/ml*				
atheter pecimen	Washout specimen	Highest final specimen	IVP	MCU
0^7	500	3×10^4	ND	ND
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$\times10^5$	500	10^4		
0^7	40	$1\ 2\times10^3$		
0^6	10	2×10^3		r(I)
0^6	5	6×10^3		*r(I)*
0	2×10^5	5×10^6		
0^4	150	10^6		
$\times10^6$	10	600		r(II)
0^7	10	300	p r	
0	10	00		
0	0	<10		r(II)
$\times10^4$	<10	30		r(I)
$\times10^4$	<10	<10	p r	r(II)
0^5	3×10^4	5×10^5		
0^5	<10	<10		
0^5	<10	<10		
0	<10	<10	ND	ND
0	<10	<10	ND	ND
$\times10^5$	<10	<10		
0	<10	<10		
0	<10	<10		
$\times10^4$	<10	<10	ND	ND
0^5	<10	500		
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25 patients cultured at least 100 000 bacteria per ml Leucocytes in uncentrifuged urine were counted in a Fuchs Rosenthal chamber All except one girl in each major group had leucocyturia (>50 per mm) and the degree did not differ between the groups

The washout procedure was performed essentially as described by Fairley et al (4) After catheterization the bladder was filled with a 0 1% lukewarm neomycin solution which was allowed to remain in the bladder for 30 min The filling had to be done quite slowly especially in the smaller children Specimens for culture were obtained when the catheter was passed (catheter specimen) after four washings with sterile saline (washout specimen) and after each of three consecutive 20 minute collecting periods (final specimens) Cultures of 0 1 and 0 003 ml urine specimens were performed on blood and Drigalski plates using pipette and quantitative loop respectively Growth of 1 000 *bacteria per ml* in one of the three final urine specimens was chosen as the lowest value consistent with an infection above the uretero-vesicular junction providing this represented at least a fivefold increase over the washout specimen

Of the 25 patients two had enterococci in the urine and 23 had *E coli* bacteria O-grouping of the *E coli* strains was performed by direct bacterial agglutination employing specific antisera (14) and in all patients bacteria of the same O-group were found in both the midstream and catheter specimens One *E coli* strain was not groupable due to spontaneous agglutination (patient no 25) The sedimentation rate was determined with a micromethod read after one hour Values ≥25 mm/h were considered to be elevated The maximal renal concentrating capacity was estimated by determining the osmolality in two consecutive urine samples obtained after at least 15 hours fluid deprivation The highest value was chosen and was compared with the normal values for this age group given by Winberg (26) i e mean −2 S D =815 mOsm/l

Serum samples obtained at the first visit and 2 weeks later were immediately frozen at −20 C The antibody determinations were done by indirect hemagglutination as described earlier (27) but employing a micropipetter (Oxford 0 025 ml) for the serial dilutions For sensitization the O antigen prepared from the urinary isolate or a standard homologous antigen was used Treatment of serum with fresh 2 mercaptoethanol was performed for measurement of the content of reduction resistant antibodies (mainly IgG antibodies) (7) The upper limit of normal titres for this age group was 1 128 for unreduced and 1 8 for reduced serum (11) Quantitative determination of C reactive protein (CRP) in serum was performed by single radial immunodiffusion (17) using specific antisera (Behringwerke AG Germany) and values >20 μg/ml were considered to indicate a parenchymal infection

Intravenous pyelography (IVP) and micturating cysto urethrography (MCU) were performed in 21 of the girls not earlier than one month after the latest infection The degree of reflux was rated in four grades where I represents reflux in a non-dilated ureter but not to the kidney pelvis II reflux into the pelvis but without dilatation of the calyces III and IV reflux with dilatation of the upper urinary tract (29)

RESULTS

Clinical and laboratory data from the 25 patients are presented in Table 1 and the results for each test summarized in Fig 1 Elevated CRP values (range 25–300 μg/ml) were found in all patients classified as having pyelonephritis In the cystitis group only one out of the 10 had measurable amounts of CRP (9 μg/ml) The sedimentation rate showed a similar pattern but with some overlapping between the two major groups The renal concentrating capacity was transiently lowered in 10 of the 14 patients with pyelonephritis while only one patient with cystitis had a low osmolality

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0^6	10	2×10^3		r(I)
0^5	5	6×10^3		r(I)
0	2×10	5×10^4		
0^6	150	10^6		
$\times10^5$	10	600		r(II)
0^7	10	300	p r	
0^7	10	700		
0^7	70	<10		r(II)
$\times10^4$	<10	30		r(I)
$\times10^4$	<10	<10	p r	r(II)
0^6	3×10^4	5×10^5		
0^5	<10	<10		
0^5	<10	<10		
0	<10	<10	ND	ND
0^7	<10	<10	ND	ND
5×10^5	<10	<10		
0	<10	<10		
0	<10	<10		
5×10^4	<10	<10	ND	ND
0^6	<10	500		
0^6	30	5×10^3		

25 patients cultured at least 100 000 bacteria per ml. Leucocytes in uncentrifuged urine were counted in a Fuchs Rosenthal chamber. All except one girl in each major group had leucocyturia (>50 per mm³) and the degree did not differ between the groups.

The washout procedure was performed essentially as described by Fairley et al. (4). After catheterization the bladder was filled with a 0 1% lukewarm neomycin solution which was allowed to remain in the bladder for 30 min. The filling had to be done quite slowly, especially in the smaller children. Specimens for culture were obtained when the catheter was passed (catheter specimen), after four washings with sterile saline (washout specimen) and after each of three consecutive 20 minute collecting periods (final specimens). Cultures of 0 1 and 0 003 ml urine specimens were performed on blood and Drigalski plates using pipette and quantitative loop respectively. Growth of 1000 bacteria per ml in one of the three final urine specimens was chosen as the lowest value consistent with an infection above the uretero-vesicular junction providing this represented at least a fivefold increase over the washout specimen.

Of the 25 patients, two had enterococci in the urine and 23 had *E. coli* bacteria. O grouping of the *E. coli* strains was performed by direct bacterial agglutination employing specific antisera (14) and in all patients bacteria of the same O group were found in both the midstream and catheter specimens. One *E. coli* strain was not groupable due to spontaneous agglutination (patient no. 25). The sedimentation rate was determined with a micromethod read after one hour. Values ≥25 mm/h were considered to be elevated. The maximal renal concentrating capacity was estimated by determining the osmolality in two consecutive urine samples obtained after at least 15 hours' fluid deprivation. The highest value was chosen and was compared with the normal values for this age group given by Winberg (26), i.e. mean −2 S D =815 mOsm/l.

Serum samples obtained at the first visit and 2 weeks later were immediately frozen at −20°C. The antibody determinations were done by indirect hemagglutination as described earlier (27) but employing a micropipetter (Oxford 0 025 ml) for the serial dilutions. For sensitization the O antigen prepared from the urinary isolate or a standard homologous antigen was used. Treatment of serum with fresh 2 mercaptoethanol was performed for measurement of the content of reduction resistant antibodies (mainly IgG antibodies) (7). The upper limit of normal titres for this age group was 1 128 for unreduced and 1 8 for reduced serum (11). Quantitative determination of C reactive protein (CRP) in serum was performed by single radial immunodiffusion (17) using specific antisera (Behringwerke AG, Germany) and values >20 μg/ml were considered to indicate a parenchymal infection.

Intravenous pyelography (IVP) and micturating cystourethrography (MCU) were performed in 21 of the girls not earlier than one month after the latest infection. The degree of reflux was rated in four grades where I represents reflux in a non-dilated ureter but not to the kidney pelvis, II reflux into the pelvis but without dilatation of the calyces, III and IV reflux with dilatation of the upper urinary tract (29).

RESULTS

Clinical and laboratory data from the 25 patients are presented in Table 1 and the results for each test summarized in Fig. 1. Elevated CRP values (range 25–300 μg/ml) were found in all patients classified as having pyelonephritis. In the cystitis group only one out of the 10 had measurable amounts of CRP (9 μg/ml). The sedimentation rate showed a similar pattern but with some overlapping between the two major groups. The renal concentrating capacity was transiently lowered in 10 of the 14 patients with pyelonephritis while only one patient with cystitis had a low osmolality

Table 1 *Clinical and laboratory findings for each patient*

ND=not done E=E coli O=O group N=non typable R=rough (spontaneously agglutinating) ec=enterococci r( )=reflux (grade) p r =parenchymal reduction

Pat no	Age yrs	Previous known UTI	Temp (°C)	CRP (μg/ml)	Sed rate (mm/h)	Conc capacity (mOsm/l)	Type of bacteria	Antibody titre reciprocal Serum Unreduced	Reduced
1	5	0	40 0	74	43	612	E O 2	8 000	16
2	6	0	40 1	58	20	938	ec	ND	ND
3	8	6	40 2	32	12	807	ec	ND	ND
4	9	6	40 1	25	14	924	E O N	256	16
5	9	1	40 1	300	35	467	E O 25	2 000	512
6	11	>10	39 5	61	28	944	E O N	64	4
7	11	1	40 3	70	25	675	E O 7	1 000	128
8	17	1	39 9	55	62	670	E O N	512	256
9	8	2	39 2	28	25	500	E O 7	2 000	128
10	10	2	39 9	260	32	932	E O N	8 000	16
11	12	2	39 7	29	36	597	E O 1	256	128
12	6	1	40 5	41	43	794	E O 8	16 000	32
13	7	0	40 1	124	55	512	E O 2	1 000	256
14	12	1	39 8	68	33	635	E O 2	512	512
15	8	1	37 3	7	27	932	E O 6	16	8
16	5	3	36 9	Neg	10	871	E O 112	32	4
17	6	1	37 8	Neg	13	797	E O 4	4	0
18	7	0	37 0	9	21	1 190	E O N	32	0
19	7	2	37 0	Neg	10	1 044	E O 2	8	2
20	8	1	37 2	Trace	4	1 072	E O 2	64	[illegible]
21	10	0	37 6	Neg	13	930	E O 25	2	0
22	12	1	36 8	Neg	4	960	E O 85	32	8
23	16	0	37 3	Neg	13	993	E O 3	32	0
24	7	1	37 2	Neg	15	925	E O 7	256	16
25	12	7	37 7	Neg	18	963	E O R	128	4

centrating capacity (8 27) or by ureteric catheterization (9) These divergent findings might be due to differences in the patient materials such as age sex and number of previous infections or by differences in diagnostic criteria employed The timing of the serum sampling in relation to the onset of the infection is also important (11 23) and measurement of antibodies in unreduced (mainly IgM) and reduced (mainly IgG) sera might be of further diagnostic value (8 24) In the present investigation we have compared different methods for determining the level diagnosis of UTI including bladder washout and serum antibody titration The material was strictly defined consisting only of girls with an acute attack of symptomatic UTI

MATERIAL AND METHODS

The patients were 25 girls age 5–17 years presenting at the emergency room of the Children s Hospital Goteborg with acute symptoms They represent a consecutive series of girls with acute urinary tract infection in whom bladder washout could be performed Since the cooperation of the children is essential in this test girls under 5 years of age have not been investigated

According to symptoms and signs a clinical diagnosis of pyelonephritis or cystitis was made Thus 14 patients (nos 1–14) were classified as having acute pyelonephritis all with high fever (>39°C) together with loin or high abdominal pain Twelve of these showed renal tenderness but only three had micturition symptoms A diagnosis of acute cystitis was made in 10 patients (nos 16–25) with burning and frequency but temperature not exceeding 38 C none of these had high abdominal or loin pain In one patient (no 15) a clinical level diagnosis could not be made She presented with acute micturition symptoms temperature of 37 3°C but only slight pain and tenderness of the left loin

In urine obtained as a midstream specimen all the

washout test teral count/ml				
neter cimen	Washout specimen	Highest final specimen	IVP	MCU
	500	3×10^4	ND	ND
0^6	600	10^4		r(II)
0^5	500	10		
	40	$1\,2\times10^3$		
	10	2×10		r(I)
	5	6×10^3		r(I)
	$?\times10^5$	5×10^6		
	150	10^5		
0^6	10	600		r(II)
	10	300	p r	
	10	700		
	0	<10		r(II)
0^4	<10	30		r(I)
0^4	<10	<10	p r	r(II)
	3×10^4	5×10^5		
	<10	<10		
	<10	<10		
	<10	<10	ND	ND
	<10	<10	ND	ND
0^5	<10	<10		
	<10	<10		
	<10	<10		
0^4	<10	<10	ND	ND
	<10	500		
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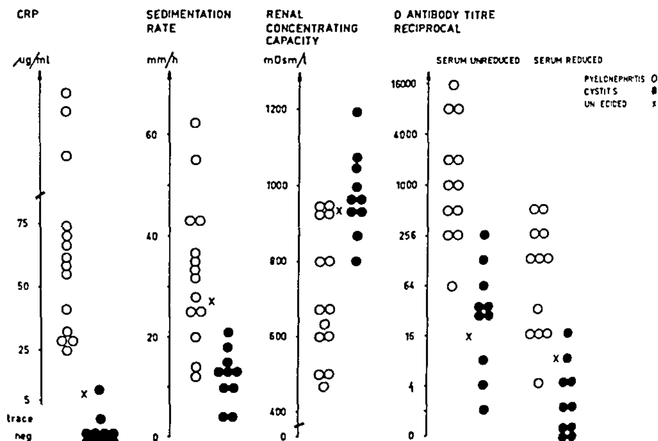


Fig 1 Outcome of determinations of CRP sedimentation rate renal concentrating capacity and antibody titres in relation to clinical diagnosis

Serum samples from the 23 patients infected with *E coli* bacteria were tested for antibodies against *E coli* O antigen Eleven of the 12 patients with pyelonephritis had elevated titres in both unreduced and reduced serum while one had normal values Among the patients with cystitis 9 had normal values while one had elevated titres of both unreduced and reduced serum (patient no 24) This patient however had had an acute febrile UTI caused by *E coli* of the same O group 5 months earlier Indeed antibody titres 2 weeks prior to the index infection were not significantly different from those reported here

In the washout test only 8 of the 14 patients with a clinical diagnosis of pyelonephritis had bacterial counts of at least 1 000 bacteria/ml in the final samples Three of the remaining girls had a 30–70 fold increase above the washout speciment but a maximal count of only 300 to 700 bacteria/ml The other three all had consistently low counts after the washout procedure Findings suggestive of bladder infection were found in all but 2 of the girls with cystitis The results from one of these (patient no 25) indicated a high infection caused by a spontaneously agglutinating strain An indefinite washout pattern with a count of 500 bacteria per ml was noted in patient no 24 whose previous infection and slightly elevated antibody titres are described above A summary of the bacterial findings in the final washout specimens is given in Fig 2 Of the 42 specimens obtained from the 14 patients with pyelonephritis only 21 had bacterial counts greater than 1 000 per ml

While the radiological investigations did not demonstrate any obstructive changes 8 patients each with clinical pyelonephritis

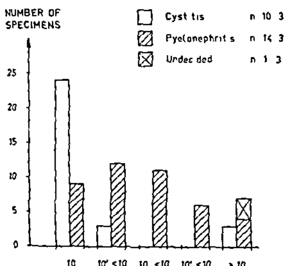


Fig 2 Number of bacteria in the final washout specimens (three in each patient) in relation to clinical diagnosis

showed abnormalities Two had renal parenchymal reduction one associated with reflux while the other 6 patients demonstrated reflux alone The 3 patients with *clinical* pyelonephritis but with washout findings indicating low infection all had radiological abnormalities as did 2 of the 3 patients with an indefinite washout pattern

The patient in whom it was not possible to make a definite clinical diagnosis (no 15) had washout values indicative of high infection slightly elevated sedimentation rate and CRP values but normal renal concentrating capacity antibody titres and radiological investigations

DISCUSSION

Reeves & Brumfitt (19) comparing different methods for localization of UTI proposed a division of tests into those giving direct or indirect evidence of involvement of the upper urinary tract According to their classification bladder washout is the only direct method utilized in this study Determination of renal concentrating capacity antibody titration and radiological investigation of the urinary tract were considered to be indirect tests It might be questioned however if there is any method other than demonstration of bacteria in kidney tissue that can give direct evidence of involvement of the renal parenchyma Due to the patchy character of pyelonephritic disease there is a risk that percutaneous biopsies will miss the affected areas and this technique has not been found useful for bacteriologic diagnosis of renal infection (1)

To arrive at an evaluation of the reliability of various methods to obtain a level diagnosis in UTI the application of a battery of different tests in patients with acute UTI seemed necessary In the present study all 14 girls with a clinical diagnosis of acute pyelonephritis had findings indicating high infection in at least three of the investigations used i e determination of CRP sedimentation rate renal concentrating capacity antibody titration bladder washout and radiological investigation Among the 10 girls with a clinical diagnosis of acute cystitis seven had findings indicative of low infection in all six tests Three of the patients with cystitis had results indicative of high infection by a single method one of these also having an indefinite washout result (patient no 24) The number of test indicative of high infection in each patient is *illustrated in Fig 3 The overall results*

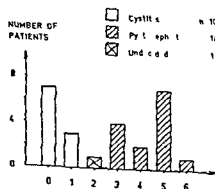


Fig 3 Number of tests in each patient indicative of high infection in relation to clinical diagnosis

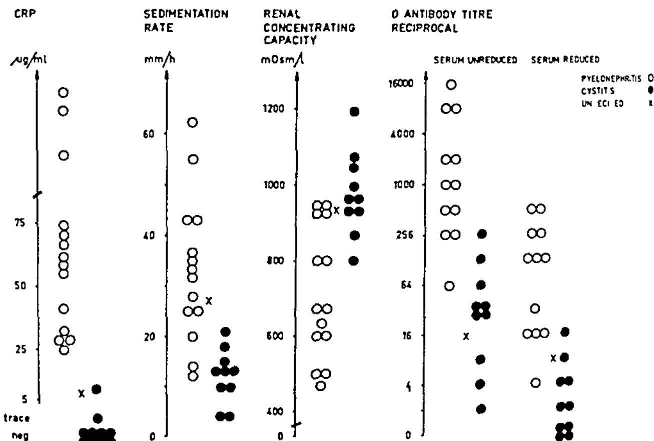


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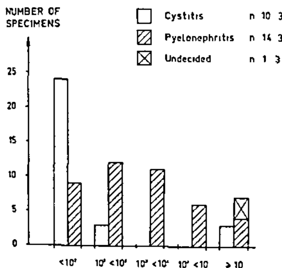


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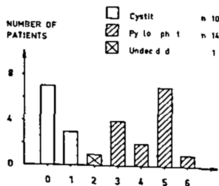


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Table 2 *Number of patients with findings indicative of high infection in relation to the number investigated (patient with undecided clinical diagnosis omitted)*

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Method	Pyelonephritis	Cystitis
CRP	14/14	0/10
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Radiology	8/13	0/7
Bladder washout	8/14	1/10

thus indicate that the clinical diagnoses assigned were highly relevant

Interpretation of the results of individual tests were generally more difficult Each method gave results indicative of high infection in from 8 to 14 of the 14 cases with pyelonephritis None of the tests indicated pyelonephritis in more than one patient with cystitis Table 2 ranks the tests according to reliability

CRP and sedimentation rate have no specific relation to kidney disease but may be increased in any severe bacterial infection (20) The high CRP levels found in all the patients with a clinical diagnosis of pyelonephritis may as does the temperature elevation merely reflect a parenchymal engagement not necessarily of the urinary tract However a simultaneous inflammation in another organ did not seem probable in the patients studied Due to its very good correlation with the clinical diagnosis and the simplicity of the method CRP determination may be a valuable test in differentiating pyelonephritis from cystitis

Only 8 of the patients with clinical pyelonephritis had results in the washout tests clearly indicating high infection Kass & Zinner (12) have pointed out that a constant outflow of bacteria into the renal pelvis is required if counting of bacteria in ureteric urine is to be used as an indication of kidney infection This however is not always the case (3, 5) and intermittent discharge of bacteria from the renal parenchyma may explain the washout findings indicative of low infection in three of the patients with a clinical diagnosis of pyelonephritis (no 12–14) In 3 other patients with acute pyelonephritis the washout findings were indefinite as all three final urine specimens contained between 100 and 1 000 bacteria per ml In fact half of the 4? final washout specimens in the 14 patients with pyelonephritis contained less than 1 000 bacteria per ml (Fig 2) If ureteric urine in children with renal infection often contains such a low number of bacteria the results of washout investigations will be difficult to interpret Our findings also emphasize the importance of a long bladder incubation time if a count of 100 000 bacteria per ml is to be used as the limit for significant bacteriuria and underlines the value of suprapubic bladder aspiration in infants and small children

Ureteric reflux may also give rise to erroneous results in the washout test if infected bladder urine retained in the ureters is emptied into the bladder after the washing has been completed A transient reflux at the time of the infection but not demonstrable by MCU a month later might explain the washout results in 2 of the patients with cystitis (nos 24 and 25) The combination of severe reflux and chronic bacteriuria does not always mean renal infection as Stamey et al (21) using ureteric catheterization found that 37% of such patients did not excrete bacteria into the upper urinary tract This on the other hand means that the radiological demonstration of a refluxing ureter does not prove that a recent UTI was of renal origin but may just as a parenchymal reduction indicate that the patient has had earlier infections Similarly the finding of elevated antibody titres may also reflect previous infections as demonstrated by our patient no 24

Three of the pyelonephritis patients (nos

and 6) had a history of many previous I Their infections were characterized by common bacterial types moderate vation of CRP and sedimentation rate as l as concentrating capacity and antibody es within or close to the normal limits ether or not this type of patients may ve an altered response to renal infections s not possible to evaluate The isolation a spontaneously agglutinating (rough) coli strain from the urine of the patient th symptoms of cystitis but washout dings of high infection (no 25) may nify an altered host response to a less ulent bacterial strain (18) These findings ough preliminary may provide a partial planation for the failure of others to monstrate elevated antibody titres in pye nephritis patients (4) The timing of serum mpling has also been shown to be im rtant (11 23)

As yet there is no evidence that any of e diagnostic methods used today are edictive of the risk of developing kidney image following UTI Possibly this risk ay depend not only on the site of infection ut also on the characteristics of the in ading organism and on the host response 5 18) The relevance of the present diag ostic methods in this respect must be valuated in prospective studies

ACKNOWLEDGEMENTS

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REFERENCES

1 Brun C Raaschou F & Eriksen K R Simultaneous bacteriologic studies of renal biopsies and urine In F H Kass (ed) *Progress in pyelophritis* Davies Philadelphia 1965 p 461

D vies A G McLachlan M S F & Asscher A W Progres i e kidney damage after non-obstru t e urinary tract infection *Br Med J* II 406 1972

3 Fairley K F & Butler H M Sterile pyuria as a manifestation of occult bacterial pyelonephritis with special reference to intermittent bacteriuria *In* P Kincaid Smith & K F Fairley (eds) *Renal infection and renal scarring* Mercedes Melbourne 1971 p 51

4 Fairley K G Grounds A D Carson N E Laird E C Gutch R C McCallum P H G Leighton P Sleeman R L & O Keefe C M Site of infection in acute urinary tract infection in general practice *Lancet II* 615 1971

5 Geisinger J F Intermittence of pyuria at the level of the renal papillae *J Urol* 25 649 1931

6 Hanson L Å Host-parasite relationships in urinary tract infections *J Infect Dis* 127 776 1973

7 Hanson L Å Holmgren J Jodal U & Kaijser B Characterization of precipitating antibodies to E coli O antigen in infants and children with acute pyelonephritis *Clin Exp Immunol* 8 573 1971

8 Hanson L Å Winberg J Andersen H J Holmgren J Jodal U & Kaijser B Significance of serum and urine antibodies in urinary tract infections in childhood *In* P Kincaid Smith & K F Fairley (eds) *Renal infection and renal scarring* Mercedes Melbourne 1971 p 117

9 Hewstone A S & Whitaker J The correlation of ureteric urine bacteriology and homologous antibody titer in children with urinary infection *J Pediatr* 74 5 0 1969

10 Hodson C J & Wilson S Natural history of chronic pyelonephritic scarring *Br Med J II* 191 1965

11 Jodal U The immune response to urinary tract infections in childhood I Serological diagnosis of primary symptomatic infection in girls by indirect hemagglutination *Acta Paediatr Scand* in press

12 Kass E H & Zinner S H Bacteriuria and renal disease *J Infect Dis* 120 27 1969

13 Kunin C M Deutscher R & Paquin A Urinary tract infection in school children an epidemiologic clinical and laboratory study *Medicine* (Baltimore) 43 91 1964

14 Lincoln K Lidin Janson G & Winberg J Resistant urinary infections resulting from changes in resistance pattern of faecal flora induced by sulphonamide and hospital environment *Br Med J III* 305 1970

15 Lindblad B S & Ekengren K The long term prognosis of non-obstructed urinary tract infection in infancy and childhood after the advent of sulphonamide *Acta Paediatr Scand* 58 25 1969

16 Manners B T B Dulake C & Grob P R Urinary tract infection in women a study from general practice *J Roy Coll Gen Practit* 19 343 1970

17 Nilsson L Å Comparative testing of precipitation methods for quantitation of C reactive protein in blood serum *Acta Path Microbiol Scand* 73 1 9 1968

18 Olling S Hanson L Å Holmgren J Jodal U

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CRP and sedimentation rate have no specific relation to kidney disease but may be increased in any severe bacterial infection (20). The high CRP levels found in all the patients with a clinical diagnosis of pyelonephritis may, as does the temperature elevation, merely reflect a parenchymal engagement not necessarily of the urinary tract. However, a simultaneous inflammation in another organ did not seem probable in the patients studied. Due to its very good correlation with the clinical diagnosis and the simplicity of the method, CRP determination may be a valuable test in differentiating pyelonephritis from cystitis.

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Ureteric reflux may also give rise to erroneous results in the washout test if infected bladder urine retained in the ureters is emptied into the bladder after the washing has been completed. A transient reflux at the time of the infection but not demonstrable by MCU a month later might explain the washout results in 2 of the patients with cystitis (nos. 24 and 25). The combination of severe reflux and chronic bacteriuria does not always mean renal infection as Stamey et al. (21) using ureteric catheterization found that 37% of such patients did not excrete bacteria into the upper urinary tract. This, on the other hand, means that the radiological demonstration of a refluxing ureter does not prove that a recent UTI was of renal origin but may, just as a parenchymal reduction, indicate that the patient has had earlier infections. Similarly, the finding of elevated antibody titres may also reflect previous infections as demonstrated by our patient no. 24.

Three of the pyelonephritis patients (nos

Acta Pædiatr Scand 64 209–214 1975

A NEW FORM OF PROLONGED TRANSIENT TYROSINEMIA PRESENTING WITH SEVERE METABOLIC ACIDOSIS

D M DANKS[1] P TIPPETT[2] and J ROGERS[3]

From the Genetics Research Unit Royal Children s Hospital Research Foundation Melbourne Australia

ABSTRACT Danks D M Tippett P and Rogers J (Genetics Research Unit Royal Children s Hospital Research Foundation Melbourne Australia) A new form of prolonged transient tyrosinemia presenting with severe metabolic acidosis Acta Paediatr Scand 64 209 1975.—Yet another form of tyrosinemia is described in a young baby who developed metabolic acidosis and ceased to grow when weaned from breast milk onto a higher protein formula Severe tyrosyluria and mild tyrosinemia cleared on a low protein diet which also corrected the acidosis However restoration of growth required a normal protein intake with very greatly reduced amounts of phenylalanine and tyrosine The metabolic fault later resolved spontaneously at about 12 months of age Mental development appears normal and liver disease was never apparent The patient and her mother both excrete quite large quantities of an unidentified peptide

KEY WORDS Dietary treatment metabolic acidosis peptiduria tyrosinemia tyrosyluria

The whole subject of tyrosinemia is extremely confused and the variable use of terms has contributed to the confusion Here *tyrosyluria* will be used to describe the excretion of *p* hydroxy phenylacetic (*p*HPAA) *p* hydroxy phenyllactic (*p*HPLA) and *p* hydroxy phenylpyruvic (*p*HPPA) acids *tyrosinemia* will be used to describe all diseases with elevated serum tyrosine as a noteworthy feature and tyrosinosis will not be used further

Transient neonatal tyrosinemia is frequent among premature babies appears to cause no symptoms and resolves spontaneously by 10–14 days (9) Remarkably high levels of serum tyrosine (and of phenylalanine) may be seen and tyrosyluria is always present

Persistent tyrosinemia and tyrosyluria with very high levels of serum tyrosine (10–25 mg per dl) but without liver disease has been described in a small number of patients most of whom have presented with mental retardation often associated with plantar and palmar hyperkeratosis and recurrent keratitis (7 8 10 11 17 20)

Liver disease has complicated the interpretation of the remaining patients reported with tyrosinemia (3 9 12 19) most of whom have come from a French Canadian isolate in Quebec Province (12) or from Sweden or Norway (1 4 5) Patients reported from other parts of the world have varied widely in severity and have included 2 patients with tyrosinemia and liver disease whose disease remitted spontaneously at 12–18 months of age after a period of dietary treatment (3 6)

The present patient differs from all previously reported cases in several important

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Lincoln K & Lindberg U The bactericidal effect of normal human serum on *E coli* strains from normals and from patients with urinary tract infections *Infection* (Munich) *1* 24 1973
19 Reeves D S & Brumfitt W Localization of urinary tract infection *In* F O Grady & W Brumfitt (eds) *Urinary tract infection* Oxford London 1968 p 53
20 Saxstad J Nilsson L Å & Hanson L Å C reactive protein in serum from infant as determined with immunodiffusion techniques II Infants with various diseases *Acta Paediatr Scand 59* 676 1970
21 Stamey T A Govan D E & Palmer J M The localization and treatment of urinary tract infections the role of bactericidal urine levels as opposed to serum levels *Medicine* (Baltimore) *44* 1 1965
22 Steele R E Leadbetter G W & Crawford J D Prognosis of childhood urinary tract infection N *Engl J Med 269* 883 1963
23 Vosti K L Monto A S & Rantz L A Host-parasite interaction in patients with infections due to *Escherichia coli* II Serologic response of the host *J Lab Clin Med 66* 613 1965
24 Vosti K L & Remington J S Host-parasite interaction in patients with infections due to *Escherichia coli* III Physicochemical characterization of O specific antibodies in serum and urine *J Lab Clin Med 72* 71 1968
25 Whitaker J & Hewstone A S The bacteriologic differentiation between upper and lower urinary tract infection in children *J Pediatr 74* 364 1969
26 Winberg J Determination of renal concentration capacity in infants and children without renal disease *Acta Paediatr Scand 48* 318 1959
27 Winberg J Andersen H J Hanson L Å & Lincoln K Studies of urinary tract infections in infancy and childhood I Antibody response in different types of urinary tract infections caused by coliform bacteria *Br Med J II* 5 4 1963
28 Winberg J Bergström T & Hanson L Å Neue Gesichtspunkte zur Harnwegsinfektion mit *E coli Monatsschr Kinderheilk 119* 596 1971
29 Winberg J Larson H & Bergstrom T Comparison of the natural history of urinary infection in children with and without vesico ureteric reflux *In* P Kincaid Smith & K F Fairley (eds) *Renal infection and renal scarring* Mercedes Melbourne 1971 p 293

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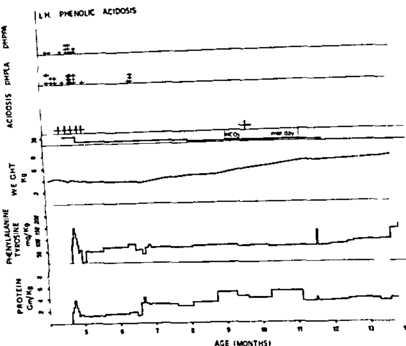


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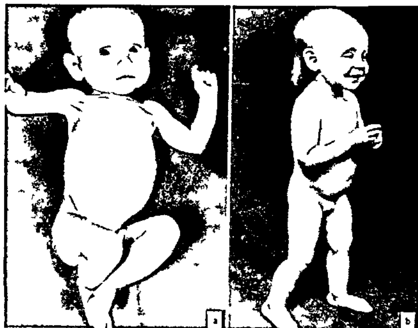


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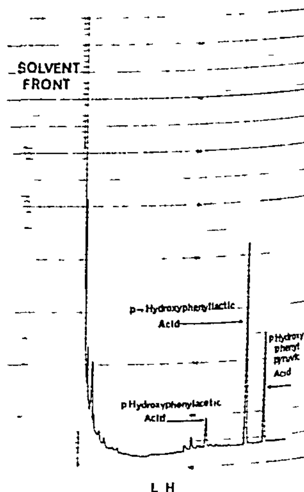


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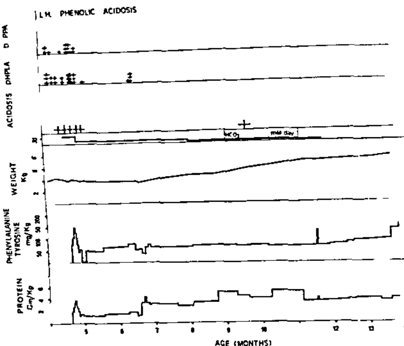


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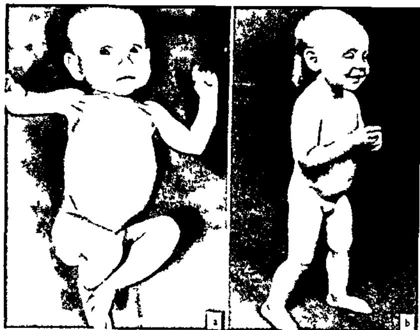


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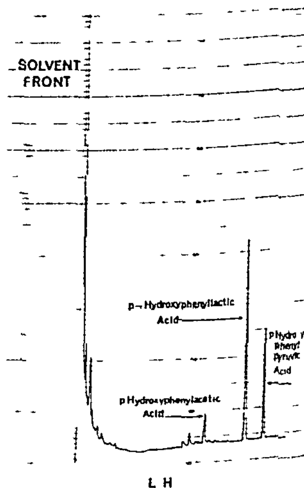


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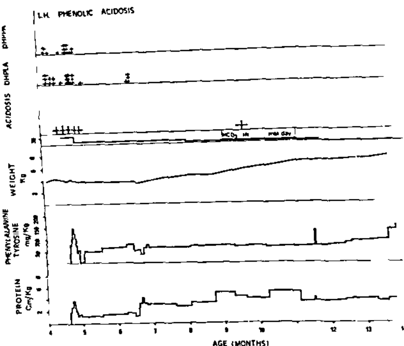


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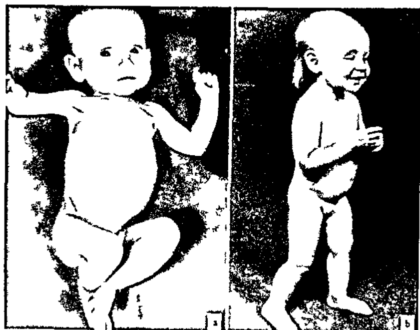


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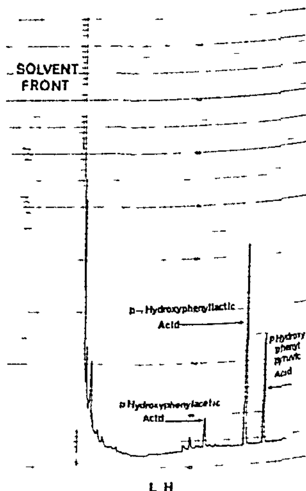


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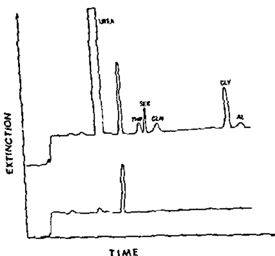


Fig 5 Ion exchange chromatography of urine from L H (upper trace) and of the unknown peptide after elution from a two-dimensional separation on paper Aminex 6 resin in a column 50×0 9 cm using 0 2 M sodium citrate buffer at pH 3 5 and a starting temperature of 30°

pound was hydrolysed by 6 M HCl for 70 hours at 110 but analysis of the residues released has still to be completed

A deuterated tyrosine loading test has recently been performed (by co-operation of Dr H Curtius of Zurich) but results will not be known for some time

DISCUSSION

It seems clear that this patient suffered a prolonged but transient disturbance of tyrosine metabolism which was effectively controlled by a diet low in phenylalanine and tyrosine The age at presentation the presence of severe metabolic acidosis and growth failure wh ch waxed and waned in parallel with changes in the tyrosyluria and the very slow resolution of the condition distinguish it from the common form of transient neonatal tyrosinemia Several premature babies with transient tyrosinemia have been noted in this laboratory to have metabolic acidosis which resolved about the same time as the tyrosyluria cleared It is just possible that this disease represents a much longer persistence of a similar metabolic fault

At no stage was there any evidence of liver disease and there is nothing to suggest brain damage Renal infection occurred but there was nothing to suggest the type of tubular damage seen in persistent tyrosinemia of the French Canadian or Scandinavian types (4 12) The mechanism responsible for the acidosis was not resolved

Recognition of this readily treatable cause of severe acidosis and growth failure is clinically important Elucidation of the precise metabolic lesion and of the relationship to the peptiduria may yet be achieved by the more detailed studies which are in progress in this laboratory and by colleagues in Zurich

ACKNOWLEDGEMENTS

Dr John Barry assisted in clinical care of the patient Miss Linda Dimech provided valuable technical assistance The co-operation of Dr H Curtius of Kinderspital Zurich Switzerland in deuterated tracer studies is acknowledged gratefully

This work was partly supported by grants from the National Health and Medical Research Council and Apex Foundation for Research into Mental Retardation

REFERENCES

1 Bodegård G Gentz J Lindblad B Lindstedt S & Zetterström R Hereditary tyrosinaemia III On the differential diagnosis and the lack of effect of early dietary treatment *Acta Paediatr Scand 58* 37 1969

2 Fell V & Pollitt R J *In* Nina Carson & Noel Raine (eds) Inherited disorders of sulphur metabolism S S I E M Symposium No 8 Churchill & Livingstone London 1971 p 232

3 Gaull G E Rassin D K Solomon G E Harris R C & Sturman J A Biochemical observations on so-called hereditary tyrosinemia *Pediatr Res 4* 337 1970

4 Gentz J Jagenburg R & Zetterstrom R Tyrosinemia An inborn error of tyrosine metabolism with cirrhosis of the liver and multiple renal tubular defects (DeToni Debre Fanconi syndrome) *J Pediatr* 66 670 1965

5 Halvorsen S Pande H Loken A C & Gjessing L R Tyrosinosis A study of 6 cases *Arch Dis Child 41* 238 1966

6 Harries J T Seakins J W T Ersser R S & Lloyd J K Recovery after dietary treatment of an infant with features of tyrosinosis *Arch Dis Child* 44 258 1969

7 Holston J L Levy H L Tomlin G A Atkins R J Patton T H & Hosty T S Tyrosinosis A patient without liver or renal disease *Pediatrics* 48 393 1971

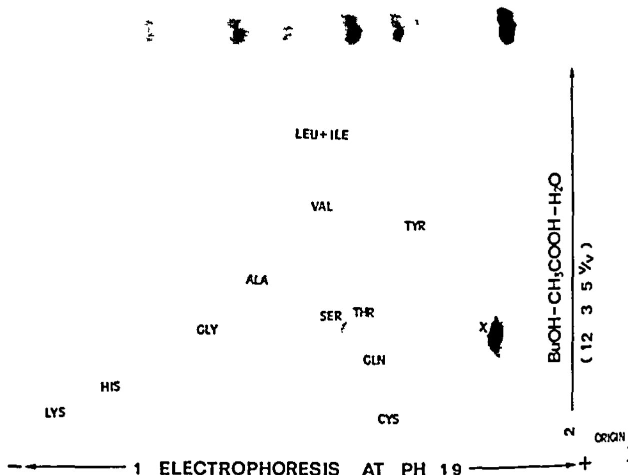


Fig 4 Two-dimensional HVE chromatography of urine The unidentified peptide is marked X

calories per kg body weight Normal acid–base balance was maintained but she failed to grow during a 5 week trial in hospital and at home Higher protein intake caused recurrence of acidosis and tyrosyluria (Fig 3) Ketonuria did not recur

Consequently she was changed to a formula (based on Albumaid XPT Scientific Hospital Supplies) containing 3 2 g protein per kg with only 50 mg/kg of phenylalanine and of tyrosine and the same caloric content Neither tyrosyluria nor acidosis recurred and she began to gain weight for the first time since 6 weeks of age Over the next 3 months she gained 2 kg and became a cheerful active baby

A brief episode of tyrosyluria and mild acidosis during a respiratory infection was the only untoward episode and she grew and became more tolerant of phenylalanine and tyrosine—90 mg per kg at 12 months 130 mg per kg at 14 months and a normal diet at 17 months

Urinary tract infection has never recurred Blood urea levels were always normal and there was no evidence of liver or kidney abnormality

At 18 months she weighed 8 7 kg and was 75 cm tall (Fig 1 *b*) She had been walking unaided for one month was very alert and had a vocabulary of 10–12 words This was regarded as acceptable development for a baby who had been so very ill for 5 months Serum amino-acid levels were normal as were urinary amino acids and phenolic acids Fig 3 charts the main features of her progress with dietary treatment

Laboratory studies

The abnormal ninhydrin staining spot present in her urine persisted without alteration throughout the dietary changes and was also found in the urine of her mother Two-dimensional HVE-chromatography of a urine sample obtained when she was well is illustrated in Fig 4 The spot stained strongly with iodo-platinate reagent (18) and weakly with sodium azide-iodine (13) Cyanide nitroprusside reagent (2) and nitroprusside alone gave negative results on a liquid urine sample These results are regarded as suggestive of a sulphur-containing peptide with an R S R configuration

Diagonal electrophoresis (7) revealed a ninhydrin positive iodo-platinate negative spot with an electrophoretic mobility less than that of the unoxidized compound Ion exchange chromatography on Aminex 6 resin using 0 2 M sodium citrate buffer at pH 3 25 showed a large unknown peak between urea and aspartic acid (Fig 5) The elution time was similar to that of methionine sulphone but the electrophoretic mobility of the unknown compound was markedly less in pH 1 9 buffer The com

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CYSTINE DEFICIENCY DURING DIETOTHERAPY OF HOMOCYSTINEMIA

CLAUDE SANSARICQ SANTOSH GARG PATRICIA M NORTON
SADASHIV V PHANSALKAR and SELMA E SNYDERMAN[1]

From the Department of Pediatrics New York University Medical Center New York USA

ABSTRACT Sansaricq C Garg S Norton P M Phansalkar S V and Snyderman S E (Department of Pediatrics New York University Medical Center New York USA) Cystine deficiency during dietotherapy of homocystinemia Acta Paediatr Scand 64 215 1975.—Cystine deficiency was inadvertently produced in a boy receiving specific dietary therapy for homocystinuria This was manifested as a loss in weight the reappearance of significant amounts of homocystine in the plasma and urine and the elevation of the plasma methionine level In addition there was a significant reduction in the level of cystine in the plasma This reduction in plasma cystine level differentiates cystine deficiency from loss of biochemical control due to failure to keep the prescribed diet The addition of cystine to the regime of this child without any other dietary modification resulted in a complete remission

KEY WORDS Homocystinuria cystine deficiency

In the first definitive study of the amino acid requirements of man Rose and his group (2 3) demonstrated that cystine was not an essential amino acid a finding that has since been confirmed by other observers as well (7) However it has become increasingly apparent that the essential nature of an amino acid may be influenced by a number of factors One of these influences is age cystine is required by at least some premature and full term infants (4 5) Another is the route of feeding the fall in plasma level in the adult during complete intravenous alimentation utilizing amino acid mixtures devoid of cystine is indicative of a special requirement at this time (6) Thirdly it has been suggested by a number of observers that cystine must be an essential amino acid for the individual with homocystinemia as a result of the metabolic block which occurs in the normal pathway of the conversion of methionine to cystine (Fig 1)

Brenton et al (1) did demonstrate negative nitrogen balance when cystine was removed from the diet of a child with homocystinemia but the effect on plasma amino acid levels was complicated by a simultaneous increase in the methionine intake as well as an inability for technical reasons to determine plasma cystine levels It is the purpose of this communication to confirm the essentiality of cystine and to report the effect of such deficiency on the plasma amino acid levels of a patient with homocystinemia

Supported by Project 317 Maternal and Child Health Services Department of Health Education and Welfare

[1] Career Scientist of the Health Research Council of the City of New York

CASE REPORT

Very little is known about the early history of this child However his neonatal course was uneventful and his Apgar score was 10 at one minute He first came under

8 Kenniwny N G & Buist N R M Metabolic studies in a patient with hepatic cytosol tyrosine aminotransferase deficiency *Pediat Res* 5 287 1971
9 La Du B N & Gjessing L R Tyrosinosis and tyrosinemia in Stanbury J B Wyngaarden J B & Fredrickson D S The Metabolic Basis of Inherited Disease McGraw Hill New York 1972
0 Louis W J Pitt D B Davies H F Metabolic studies in a patient with tyrosinosis *Aust N Z J Med* 4 281 1974
1 Medes G New error of tyrosine metabolism tyrosinosis Intermediary metabolism of tyrosine and phenylalanine *Biochem J* 26 917 1932
2 Scriver C R Partington M & Sass Kortsak A Conference on Hereditary Tyrosinemia *Can Med Assoc J* 97 1045 1967
3 Smith I (ed) Chromatographic and Electrophoretic Techniques Heinemann London 1969 3rd edition Vol 1 p 122
4 Spackman D H Stein H W Moore S Automatic recording apparatus for use in the chromatography of amino acids *Anal Chem* 30 1190 1958
5 Tippett P A & Danks D M Screening for aminoaciduria (i) a critical evaluation of 4 techniques (ii) a survey of a mentally retarded population using high voltage paper electrophoresis *Aust Paediatr J* 8 174 1972
16 Tippett P Danks D M & Dimech L Detection of life threatening inborn errors of metabolism during infancy *Aust Paediatr J* 1974
17 Wadman S K Van Sprang F J Maas J W & Ketting D An exceptional case of tyrosinosis *J Ment Defic Res* 12 269 1968
18 Wilcken B Turner B & Brown D A Detection of abnormal sulphur containing amino acid excretion in a mass urine screening programme *Med J Aust* 1 1193 1972
19 Yu J S Walker Smith J A & Burnard E D Neonatal hepatitis in premature infants simulating hereditary tyrosinosis *Arch Dis Child* 46 306 1971
20 Zaleski W A & Hill A Tyrosinosis a new variant *Can Med Assoc J* 108 477 1973

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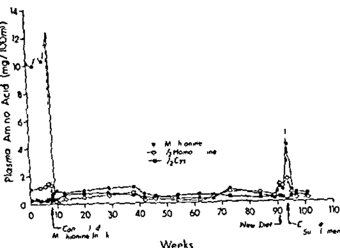


Fig 2 Effect of various dietary regimens on plasma amino acid levels

els There was no change in either blood values or the child s clinical condition after 2 weeks However definite manifestations of cystine deficiency were apparent 3 weeks after this regimen was instituted There was a weight loss of 0 8 kg the plasma methionine had risen to 4 mg/100 ml the plasma 1/2 homocystine level was over 2 mg/100 ml while the plasma 1/2 cystine level had dropped to 0 08 mg/100 ml There were no changes in any of the other plasma amino acid levels A supplement of 32 mg/kg/day of cystine was started immediately without any other dietary change This resulted in a complete reversal of the biochemical abnormalities and the weight loss was immediately regained (Fig 2)

DISCUSSION

The child manifested the usual signs of an amino acid deficiency—loss in weight and a depression of the plasma level of the deficient amino acid In addition there were plasma deviations which occurred as a result of the metabolic block—an elevation of the plasma methionine and homocystine levels and also the urinary excretion of appreciable amounts of homocystine These latter deviations can be explained as the result of breakdown of tissues having a normal complement of methionine so that the end result is similar to feeding a normal diet The biochemical and clinical response to an increase in cystine intake the only dietary alteration effected at this time confirmed the cystine deficiency state Elevations of methionine and homocystine are of course also observed as a result of loss of dietary control though this can be differentiated from cystine deficiency by determining the level of cystine in the plasma

This complication in the therapy of this patient once again emphasizes the meticulous care that must be taken whenever dietary therapy is instituted in the management of a metabolic disease The physician must not only prescribe the proper diet but he must also be certain that there is dependable quality control of the special formulas that are employed [1]

REFERENCES

1 Brenton D P Cusworth D C Dent C E & Jones E E Homocystinuria clinical and dietary studies *Q J Med* 139 325 1966
2 Rose W C The amino acid requirements of man *Fed Proc* 8 546 1949
3 Rose W C & Wixom R L Amino acid requirements of man Sparing effect of cystine on methionine requirements *J Biol Chem* 216 763 1955
4 Snyderman S E Protein and amino acid requirements of the premature infant *In Metabolic Processes in the Foetus and Newborn Infant* H E Stenfert Kroese N Y Leiden 1971
5 Snyderman S E Unpublished data

[1] This deficiency was immediately called to the attention of the manufacturer who instituted the necessary procedure to control the amino acid composition of the diet

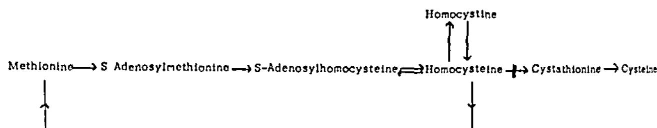


Fig 1 A simplified version of the metabolism of methionine demonstrating the block in homocystinemia

medical observation at 3 years of age when he was removed from his home because of neglect and placed in an institution On admission his physical condition was good his height and weight were within normal limits but he was obviously retarded and in addition he had not developed any intelligent speech

This boy was referred to our center at 4 years of age as a result of a positive urine screening test for cystine At this time his physical examination revealed that he was unusually large for his age both his height and weight were well over the 97th percentile Other findings of note were low set flattened ears a mild kyphosis (also observed by roentgenographic examination) and a soft systolic murmur Examination of the eyes including slit lamp did not reveal any abnormalities The presence of retardation was quite apparent In addition hyperactivity clumsiness and a lack of coordination were observed during the hospitalization The electroencephalogram was abnormal the bilateral dysfunction was consistent with a seizure disorder Retardation was confirmed by psychologic testing he achieved an I Q Score of 74 on the Merrill Palmer and one of 53 on the Stanford Binet The suspected diagnosis of homocystinemia was confirmed by finding a level of 13 2 mg/100 ml of methionine (normal 0 2–0 4) and 1 1 mg/100 ml of half homocystine in the plasma Urinary excretion of homocystine was 11 5 mg/g of creatinine Massive doses of pyridoxine 500 to 1000 mg daily supplemented with 20 mg of folic acid and 2 mg of cyanocobalamin had no effect on his plasma amino acid levels Therefore after one month on this form of treatment we considered him to be non responsive to vitamin therapy and decided to try methionine restriction

Treatment

Dietary therapy was initiated by supplying the protein of the diet in the form of an L amino acid mixture free of methionine (Table 1) His methionine requirement was met by the small amounts of protein contained in the permitted low protein foods The child accepted the diet surprisingly well and biochemical control was rapidly achieved and this was in turn followed by slow clinical improvement There was less hyperactivity he was less clumsy and his improved coordination allowed him to do manual tasks that had previously been impossible for him He began to speak and his vocabulary gradually increased His general behavior improved he was much more tractible and he was able to attend an educable class in school Subsequent testing revealed a gradual improvement in his psychologic tests

After he had been on this regimen for over a year and a half we were offered the use of a preparation which had been formulated for the treatment of this disease According to the list of contents supplied with this new product it was free of methionine but contained cystine in an amount quite similar to the mixture we had been using It also contained carbohydrate minerals and vitamins Our usual procedure with any new amino acid mixture is to check on the presumed content by an analysis employing ion exchange column chromatography This was done with this material and the results very closely approximated the claimed contents with one striking exception there seemed to be a complete absence of cystine! This finding so surprised us that we considered the most likely possibility to be some error in our technique that perhaps the cystine was bound in such a way to one of the other constituents of this formula that it was not possible to detect it Accordingly we decided to try the new product but to keep the child under close observation One week later his weight was maintained the plasma cystine level was in the same range as previously and there was a moderate increase in his plasma methionine and homocystine lev

Table 1 *The composition of the amino acid mixture*

The patient was given 1 5 g/kg/day This provided 3? mg/kg of cystine daily

	Constituents (grams)
L alanine	2 67
L arginine	4 58
L aspartic acid	8 78
L-cystine	2 14
L glutamic acid	17 56
Glycine	3 72
L histidine	1 76
L isoleucine	6 11
L leucine	11 76
L lysine	7 10
L phenylalanine	4 89
L proline	6 11
L serine	5 34
L threonine	4 58
L tyrosine	4 58
L tryptophan	1 68
L valine	6 64
	100

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IATROGENIC OSTEOMALACIA IN EPILEPTIC CHILDREN

A Controlled Therapeutic Trial

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From the Departments of Clinical Chemistry Clinical Physiology Paediatrics and Neurology Glostrup Hospital 2600 Glostrup and the Department of Clinical Physiology Aalborg Sygehus Syd 9000 Aalborg Denmark

ABSTRACT Christiansen C Rødbro P and Thøger Nielsen C (Departments of Clinical Chemistry Clinical Physiology Paediatrics and Neurology Glostrup Hospital 2600 Glostrup and Department of Clinical Physiology Aalborg Sygehus Syd 9000 Aalborg Denmark) Iatrogenic osteomalacia in epileptic children A controlled therapeutic trial Acta Paediatr Scand 64 219 1975.—Bone mineral content (BMC) in the forearms (related to total body calcium) was measured for a controlled therapeutic trial in 25 epileptic children on long term treatment with phenytoin and in 22 normal children before and during treatment with vitamin D or placebo In the epileptic children hypocalcaemia and elevated serum alkaline phosphatase was found in 20% and 16% respectively The group of epileptic children treated with vitamin D_2 (2000 IU daily) for 3 months showed a significant increase in bone mineral content 5% on average The epileptic children treated with placebo showed a significant decrease 2% on average while the normal children treated with vitamin D or placebo showed no change in bone mineral content. The results indicate a possible benefit of prophylactic vitamin D treatment in epileptic children

KEY WORDS Bone mineral content, total body calcium epileptic children osteomalacia vitamin D

Within recent years a number of reports have shown a disturbance of vitamin D metabolism in epileptics treated with anticonvulsant drugs (3 4 8 11 13 21 22 23) This disturbance appear to be due to an induction of liver enzymes concerned with the metabolism of vitamin D and the phrase Anticonvulsant Osteomalacia has been coined for this condition (9) The pathological findings comprise the following features in adult patients Hypocalcaemia (3 11 13 21) elevated serum alkaline phosphatase (3 13 21) a depressed bone mineral content (BMC) (3 4 17 22) bone biopsy findings characteristic of osteomalacia (8) and a lowered serum concentration of 25 OH D_3 (11 23) A few reports have described similar biochemical changes in epileptic children on long term anticonvulsant treatment (13 14 16 18) Some authors have tried to evaluate the frequency of osteopenia from X ray examinations in children (1 14 16 18) but the reports from various centres do not accord too well possibly due to the qualitative nature of the radiological investigation

Photon absorptiometry has proved valuable to study the effect of vitamin D in adult epileptics (3 4 6 22)

We report here a controlled therapeutic trial in epileptic children in which photon absorptiometry was used to evaluate the effect of vitamin D upon total body calcium as estimated from BMC (4 6 7)

6 Stegink L D & Den Besten L Synthesis of cys teine from methionine in normal adult subjects effect of route of alimentation *Science 178* 514 1972
7 Swendseid M D Williams I & Dunn M S Amino acid requirements of young woman based on nitrogen balance data I The sulfur containing amino acids *J Nutr 58* 495 1956

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Within recent years a number of reports have shown a disturbance of vitamin D metabolism in epileptics treated with anticonvulsant drugs (3 4 8 11 13 21 22 23) This disturbance appear to be due to an induction of liver enzymes concerned with the metabolism of vitamin D and the phrase Anticonvulsant Osteomalacia has been coined for this condition (9) The pathological findings comprise the following features in adult patients Hypocalcaemia (3 11 13 21) elevated serum alkaline phosphatase (3 13 21) a depressed bone mineral content (BMC) (3 4 17 22) bone biopsy findings characteristic of osteomalacia (8) and a lowered serum concentration of 25 OH D_3 (11 23) A few reports have described similar biochemical changes in epileptic children on long term anticonvulsant treatment (13 14 16 18) Some authors have tried to evaluate the frequency of osteopenia from X ray examinations in children (1 14 16 18) but the reports from various centres do not accord too well possibly due to the qualitative nature of the radiological investigation

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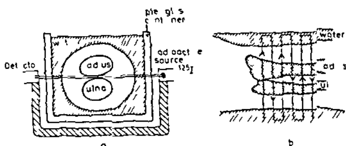


Fig 1 Principle in measurement of bone mineral content by direct photon absorptiometry on forearm (*a*) Section through system showing plexiglass container and U shaped holder with radioactive source detector (*b*) System seen from volar surface showing scanning movements of source and detector

on average 104 and 488 respectively in the Copenhagen area (Danish Institute of Meteorology personal communication)

The same procedure was followed in the 25 epileptic children and in the 22 normal children (group A) At time t (start of study) BMC was determined and blood samples were drawn for determination of serum calcium and serum alkaline phosphatase levels and furthermore in the epileptic patients for determination of the serum concentration of phenytoin After 3 months treatment (with vitamin D_2 or placebo) these measurements were repeated (at time t)

After the initial BMC measurement the epileptic children and group A of normal children were allocated to a vitamin-D-treatment group or a placebo treatment group using random sampling numbers (7) The number of subjects in each group is shown in Fig 3 The first group was treated with calcium Sandoz® 500 mg per day and vitamin D_2 (2 000 IU daily) both drugs given by mouth and the second group was treated with calcium Sandoz® 500 mg per day and placebo During the whole study the children of both groups were derived of vitamin D supplies except what is found in food

In connection with the final examination (at time t_1) the epileptic children answered a simple questionnaire asking whether after 3 months treatment they generally felt *better the same* or *worse*

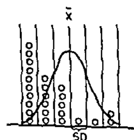
Standard statistical tests were used (7)

RESULTS

The distribution of individual serum values of calcium and alkaline phosphatase for the 25 epileptics are given in Fig 2 which shows the values in relation to mean and standard deviation of corresponding normal sex and age group As the serum alkaline phosphatase values showed a skew distribution all alkaline phosphatase values from epileptics and from control group B are calculated as the logarithms of the actual values The proportion of patients having values higher than normal mean was calculated for each of the biochemical parameters (for serum calcium 16% for alkaline phosphatase 88%)

The difference between these proportions and the proportions found in the control was highly significant ($p<0.001$ using test for difference between proportions)

The effect of treatment upon the biochemical parameters is given in Table 3 For both vitamin D and placebo groups a significant increase in serum was found during the study while no



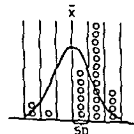


Fig 2 Distribution of serum calcium (above) and serum alkaline phosphatase (below) in 25 *epileptic children* compared with *corresponding* normal mean and standard deviations (Values for alkaline phosphatase after logarithmic transformation)

Table 1 *Age dose of phenytoin serum levels of phenytoin and duration of treatment in 25 epileptic children*

Treatment	No of patients	Age (years)		Phenytoin dose (mg/kg)		Serum level of phenytoin (mg/l)				Duration of phenytoin treatment (years)	
						Before trial		After trial			
		Mean	Range	Mean	Range	Mean	Range	Mean	Range	Mean	Range
Vitamin D	14	9 4	7–13	6 6	5 5–8 6	8 5	2–19	8 2	0–16	2	1–7
Placebo	11	10 6	7–14	6 1	4 1–9 3	8 6	3–14	7 8	2–16	2	1–4

PATIENTS AND METHODS

Epileptic patients

Of the epileptic out patients who were regularly checked at the Department of Paediatrics at Glostrup Hospital 25 children were selected for the study They all fulfilled the following criteria They were between 7 and 14 years of age had been treated with phenytoin for at least one year had no story of digestive or renal disorders and all had normal serum values of creatinine and urea Data for age anticonvulsant dose duration of treatment and serum levels of anticonvulsant drugs are given in Table 1

Control subjects

Two groups of controls were studied None of the children had a history of digestive or renal diseases and all had normal serum values for creatinine and urea Group A comprising 22 healthy children between 7–14 years of age served as control group for the controlled therapeutic trial (see below) Group B comprising 101 healthy children between 7 and 14 years of age served as control group for serum calcium and serum alkaline phosphatase values The sex and age distribution together with the biochemical values are given in Table 2

Methods

The bone mineral content was determined by direct photon absorptiometry on both forearms (Fig 1) Antebrachium is fixed in a plexiglass container filled with distilled water The source of radiation (25 mCi ^{125}I) and the detector are fixed in a holder at each side of the bones to be examined A mechanical scanner displaces the detector and the source perpendicular to the longitudinal axis of the bones The transmission of the photons through the bones depends on the bone mineral content Because the linear attenuation coefficients of plexiglass distilled water and soft tissue are almost identical the measurement is independent of the amount of soft tissue There exists a direct relation between the absorption of photons from ^{125}I and BMC in our version of the method (3 7) the BMC is expressed in arbitrary units as a mean value of six scans from each forearm The coefficient of variation of duplicate measurements in the same patient on different days is of the order 2–4 % BMC of the forearm is fairly well correlated to the body s total calcium content (7 20)

Blood samples were taken from all patients and the control subjects in group B without stasis Serum calcium was determined by an atomic absorption spectrophotometer (Perkin Elmer 403) and serum alkaline phosphatase was determined by the method of March et al (19) The coefficients of variation of duplicate measurements were 1 0 % and 3 2 % respectively

The concentration of phenytoin was measured by the method of Larsen et al (15)

Procedure

The therapeutic trial was conducted from May 1973 to August 1973 The number of sunshine hours in the 4 month period from January to April and from May to August was

Table 2 *Distribution of sex and age together with serum calcium and serum alkaline phosphatase levels in 101 healthy children*

Age	Boys					Girls				
	No of subjects	Serum calcium (mg/l)		Serum alkaline phosphatase (K A u /100 ml)		No of subjects	Serum calcium (mg/l)		Serum alkaline phosphatase (K A u /100 ml)	
		Mean	S D	Mean	S D		Mean	S D	Mean	S D
7–8	10	98 0	2 0	21 3	5 1	8	97 6	1 3	18 2	6 3
9–10	11	96 7	1 5	19 5	5 7	10	100 0	2 6	21 4	3 6
11–12	17	102 2	2 8	22 1	5 0	16	100 8	2 4	18 8	5 2
13–14	14	101 3	3 4	24 2	4 1	15	99 8	3 5	11 4	4 8

Our present finding that serum calcium during the study which took place from May to August increased in both the vitamin D and the placebo groups indicates that this was due to an extraneous influence and not to the given treatment of vitamin D_2 or calcium

In adult epileptics a similar dose of vitamin D_2 as given here implies an increase in BMC of 3–4% (3) comparable to our findings of 4–5% The fact that mean BMC values fell significantly in the placebo group during the study probably means that the children were deprived of vitamin D supplies except for those found in food This amounts only to approximately 200 IU daily for an adult (12) The findings are consistent with the previously reported phenomenon that anticonvulsant osteomalacia develops rather quickly in the course of a few months after institution of anticonvulsant therapy (22) In the control groups of normal children the BMC values did not change during the study

A number of reports during recent years have emphasized the importance of diagnosing anticonvulsant osteomalacia It is even possible that the number of the epileptic fits falls when vitamin D_2 is given (5) It is an open question in which form vitamin D should be given and the optimal dose has not yet been found (4 6) but it seems certain that epileptic children who must be considered as a group at risk should be given supplementary vitamin D treatment in some form or other

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REFERENCES

1 Berger G & Munde B Knochenstrukturveränderungen bei antikonvulsiver Langzeitbehandlung *Dtsch Gesundheitsw* 15 1549 1970

Bradford Hill A *Principles of medical statistics* The Lancet Limited London 1967 8th ed

3 Christiansen C Rødbro P & Lund M Incidence of anticonvulsant osteomalacia and effect of vitamin D Controlled therapeutic trial *Br Med J IV* 695 1973

4 Christiansen C & Rødbro P Initial and maintenance doses of vitamin D_2 in the treatment of anticonvulsant osteomalacia *Acta Neurol Scand* (in press)

5 Christiansen C Rødbro P & Sjö O Anticonvulsant action of vitamin D in epileptic patients? A controlled pilot study *Br Med J II* 258 1974

6 Christiansen C Rødbro P Munck O & Munck O Different action of vitamin D_2 D_3 and 25 OH D_3 in anticonvulsant osteomalacia To be published 1974

7 Christiansen C & Rødbro P To be published 1974

8 Dent C E Richens A Rowe D J F & Stamp T C B Osteomalacia with long term anticonvulsant therapy in epilepsy *Br Med J IV* 69 1970

9 Editorial Anticonvulsant osteomalacia *Lancet II* 805 1972

10 Gupta M M Round J M & Stamp T C B Spontaneous cure of vitamin D deficiency in Asians during summer in Britain *Lancet I* 586 1974

11 Hahn T J Hendin B A Scharp C R & Haddad J G Effect of chronic anticonvulsant therapy on serum 25 hydroxycalciferol levels in adults *N Engl J Med* 287 900 1972

12 Hansen K M Kostundersøgelser 1971 *Husholdningsrådets tekniske meddelelser* 2 16 1973

13 Hunter J Maxwell J D Stewart D A Parsons V & Williams R Altered calcium metabolism in epileptic children on anticonvulsants *Br Med J IV* 202 1971

14 Kruse R Osteopathien bei antiepileptischer Langzeittherapie (Vorläufige Mitteilung) *Monatsschr Kinderheilkd* 116 378 1968

15 Larsen N E Naestoft J & Hvidberg E Rapid routine determination of some anti-epileptic drugs in serum by gas chromatography *Clin Chim Acta* 40 171 1972

16 Lifshitz F & Maclaren N K Vitamin D-dependent rickets in institutionalized mentally retarded children receiving long term anticonvulsant therapy I A survey of 288 patients *J Pediatr* 83 612 1973

17 Linde J Hansen J M Siersbaek Nielsen K & Fuglsang Frederiksen V Bone density in patients receiving long term anticonvulsant therapy *Acta Neurol Scand* 47 650 1971

18 Maclaren N K & Lifshitz F Vitamin D-dependency rickets in institutionalized mentally retarded children on long term anticonvulsant therapy II The response to 25 hydroxycholecalciferol and vitamin D *Pediatr Res* 7 914 1973

19 March W H Fingerhut B & Kirsch E Adaption of an alkaline phosphatase method for automatic colorimetric analysis *Clin Chem* 5 119 1959

20 Mazess R B Estimation of bone and skeletal weight by direct photon absorptiometry *Invest Radiol* 6 52 1971

21 Richens A & Rowe D J F Disturbance of calcium metabolism by anticonvulsant drugs *Br Med J IV* 73 1970

22 Rødbro P Christiansen C & Lund M Develop-

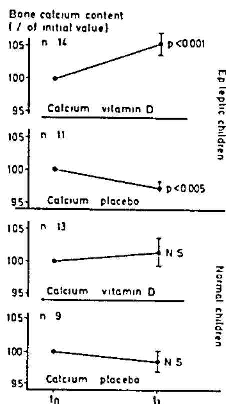


Fig 3 Bone mineral content (per cent of initial value) before and after trial in 25 epileptic children and 22 normal children

change was seen for serum alkaline phosphatase

The effect of treatment on BMC is shown in Fig 3 The BMC value increased significantly in the vitamin D treatment group and fell significantly in the placebo group of the epileptic children while no change was observed for the healthy children in control group A

The serum calcium and serum alkaline phosphatase levels were not correlated to dose or duration of anticonvulsant treatment Similarly no correlation was found between the effect of treatment evaluated from percentual increase in BMC and parameters as age body weight dose and duration of phenytoin therapy and initial values of serum calcium and serum alkaline phosphatase

In reply to the questionnaire five out of the 14 children on vitamin D treatment answered after completion of the study that they generally felt better 9 felt generally the same and none felt generally worse The corresponding figure of the 11 patients in the placebo group were 0 10 and 1

DISCUSSION

Our results confirm the findings of Hunter et al (13) that epileptic children on anticonvulsant therapy show hypocalcaemia and elevated serum alkaline phosphatase In a recent publication (6) we have demonstrated that vitamin D and D_3 act in a different manner in grown up epileptics Vitamin D increases BMC but leaves the serum calcium levels unaffected whereas vitamin D_3 increases serum calcium but has no effect upon BMC Gupta et al (10) have shown a spontaneous cure of vitamin D deficiency in Asians during summer in Britain as both serum calcium and 25 OH D_3 increased during the summer months This reflects the importance of ultraviolet irradiation which increases the synthesis of vitamin D_3 We have previously shown (3 6) that serum calcium in epileptics is unchanged during treatment with calcium when it is given in the winter months

Table 3 *Difference from initial values of serum calcium and serum alkaline phosphatase in 25 epileptic children after treatment for 3 months with calcium plus vitamin D or calcium plus placebo*

		Serum calcium		Serum alkaline phosphatase	
Treatment	No of patients	Mean difference (mg/l) from initial value	Significance of difference	Mean difference (K A u /100 ml) from initial value	Significance of difference
Vitamin D	14	3 1	$p<0$ 02	−1 0	N S
Placebo	11	4 2	$p<0$ 001	−0 4	N S

N S =Not significant ($p>0$ 05)

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HEALTH AND BEHAVIOUR IN FOUR YEAR OLD CHILDREN

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ABSTRACT Kohler E M and Kohler L (Department of Paediatrics University Hospital Lund Sweden) Health and behaviour in four year-old children Acta Paediatr Scand 64 225 1975.—A health control of an unselected population of 2447 4-year-old children included a thorough somatic examination as well as an analysis of child upbringing practice and problems The relation between the children s physical health as defined by presence or absence or functionally important health problems and the children s behaviour as reported by their mothers was elucidated On the whole children with health problems were not perceived as more troublesome although children with some special kinds of disturbances e g neurological and dental constituted problems in certain areas e g toilet training and hyperactivity The use of blame as a method of upbringing was very frequent and especially frequent in children with dental defects and visual disturbances The perception of behaviour problems and the use of methods in upbringing were the same in children with newly detected health problems as in children with previously known health problems The implications for the Child Health Service are to identify these risk-groups to advice and support them in order to reduce parent–child conflicts

KEY WORDS Preschool children child rearing physical health behaviour

Child upbringing is an interaction between the child and its environment The child s various responses to parental demands in daily routine moulds the parent s attitudes and actions towards their offspring to a certain extent (1 12 13 14 16 34 37) By educational means the parents try to adapt their upbringing to the perceived possibilities and skills of their child This adaptive process is sometimes an unconscious one and is of course limited by the actual knowledge of upbringing and the educational material available to the parents (10) Children s behavioural deviations of affective and social nature may be regarded as results of disturbances in this interaction between child and environment

The child s physical health plays an important role in the process of upbringing (5 7 30 33 39) Illnesses like measles pneumonia or frequent upper respiratory infections are not to be regarded as isolated physical episodes they may also imply physical emotional and social disturbances affecting the whole family during and some time after the illnesses (7) Chronic illnesses like major auditory and visual impairments and neurological disturbances may provoke special parental attitudes from consideration and understanding to overprotection or rejection (6 8 25 38)

In addition children with minor physical handicaps unrecognised by the parents may provoke other attitudes from the parents instead of being thought of as affected by neurological or visual disorders the children are regarded as disobedient naughty and negligent and also treated accordingly (11 31 32 33)

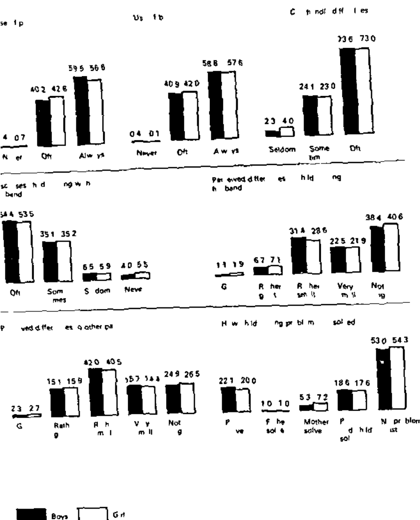
The purpose of the present study is to fur

ment of anticonvulsant osteomalacia in epileptic patients on phenytoin treatment *Acta Neurol Scand* (in press)

23 Stamp T C B Round J M Rowe D J F & Haddad J G Plasma levels and therapeutic effect of 25 hydroxycholecalciferol in epileptic patients taking anticonvulsant drugs *Br Med J IV* 9 1972

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F g 1 Frequency distribution (in per cent) for seven questions on child rearing answered by the mothers Separate presentation for boys and girls

capping disorders Thus we think that children with minimal brain dysfunction constitute problems for *them* selves and their surrounding and that better prospects for their adjustment and function are reached by their recognition and treatment Likewise we think it is im *portant that children* should have optimal use of their main information media and that a suboptimal funct on be it a *functional* amblyopia or a significant hearing loss may limit or distort true conception of their sur roundings and cause unnecessary strain

Also we th nk that *extensive and deep* caries here called emergency cases contribute *to long term* suboptimal health and can be painful debilitating and expensive Therefore al o this kind of disorders should be regarded as important for the children How ever it is qu te obvious *that the* most handicapping conditions were detected long before the age of 4 years e g cerebral palsy hydrocephalus severe hearing loss *muscular* dystrophia congenital nystagmus (18 19 20 24) Generally therefore newly detected health prob lems were less of a handicap than those previously known

The prevalence of such functionally important health problems was 4 9% found in 21 6% of the children The distribution on groups of diagnoses are shown in Table 1 The children and their diagnoses are discussed in detail elsewhere (24)

Uph inging

From the questionnaire used in the health control three groups of *questions* were used in analysis of upbringing (17) The first group of questions concerned more gen eral aspects of child rearing i e general use of praise

Table 1. *Functionally important health problems in 2 447 four year old children*

	Previously known		Newly detected		Sum	
	n	%	*n*	%	*n*	%
Neurological	13	0.5	54	2.2	67	2.7
Other Somatic	55	2.2	22	0.9	77	3.1
Cardiac	14	0.6	1	0.1	15	0.6
Other Paediatric	13	0.5	12	0.5	25	1.0
Surgical	18	0.7	9	0.4	27	1.1
Orthopaedic	10	0.4	0	0	10	0.4
Dental			204	8.3	204	8.3
Visual	67	2.7	154	6.3	221	9.0
Auditory	2	0.1	39	1.6	41	1.7
Sum	137	5.6	473	19.3	610	24.9

ther elucidate the relationship between somatic health in a broad sense and problems of child upbringing perceived by parents in an unselected population of 4 year old children.

According to the discussion above, the following hypotheses may be formed regarding this relationship:

1. Mothers of children with chronic physical health problems perceive more behavioural problems among their children than do other mothers.

2. Mothers of children with previously known chronic physical health problems more seldom punish and rebuke their children and more frequently use praise.

3. Mothers of children with previously unknown chronic physical health problems have perceived more behavioural problems during the children's first 4 years and also have perceived less success in their upbringing than have mothers of children with previously known chronic physical health problems.

MATERIAL

Since 1967 a general health control of 4 year old children has been organized in the city of Lund (50 000 inhab.) and since 1968 in the community of Dalby (8 500 inhab.) in the vicinity of Lund. All children at the age of 4 years living in these areas were selected from the county population register. Children living temporarily in the areas but registered elsewhere were excluded. There was a total of 2 573 4 year olds: 2 296 living in Lund 1967–69 and 277 in Dalby 1968–69. Altogether 2 447 children (1 272 boys and 1 175 girls) (95.1%) participated in the study (24).

METHODS AND BACKGROUND DATA

The children were invited to participate by a letter to their parents. The invitation was accompanied by questionnaires regarding parental opinion on problems of rearing and of the child's behaviour in some specific every day situations like going to bed, eating, playing (17).

Somatic health

A comprehensive physical examination was performed by a paediatrician (L. K.) and children with physical health problems were referred for further evaluation to the respective departments of the University Hospital of Lund (18, 23). The vision and audiology screening were carried out by specially trained nurses. Children who failed the tests were examined by an ophthalmologist or an audiologist and a clinical diagnosis was established (19, 20).

The dental study was performed by a clinical and roentgenological examination. Children with need of pulpotomy or extraction of 2 or more molars in combination with 15 or more decayed surfaces were designated as "emergency cases" (21).

The clinical findings of children with health problems newly detected or previously known were also evaluated in terms of functional importance for the child. A *functionally important health problem* was defined as a disorder which is likely to have a significant and prolonged impact on the child's health and development or to hamper the full exploitation of its environment at present or in the future (24). This concept includes not only children with well recognized handicapping disorders like gross cerebral palsy and severe hearing loss but also minor physical deviations like minimal brain dysfunction, more pronounced errors of refraction and bad dental status. In an economically and socially well developed society with an advanced system of medical care there are resources at hand even for minor handi

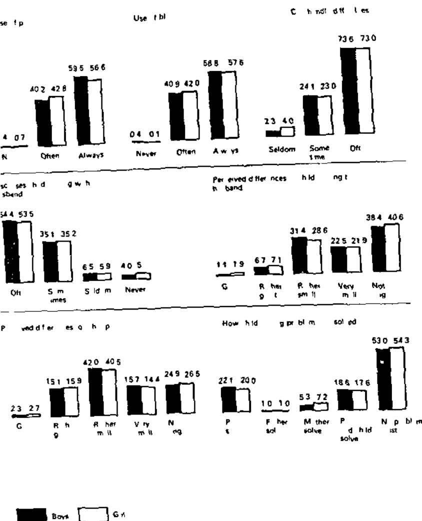


Fig 1 Frequency distribution (in per cent) for seven questions on child rearing answered by the mothers Separate presentation for boys and girls

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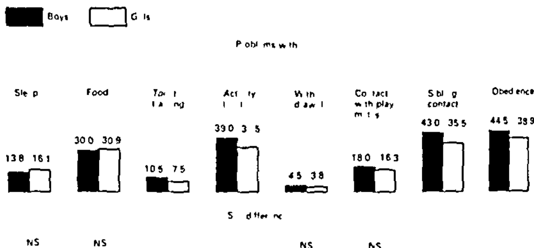
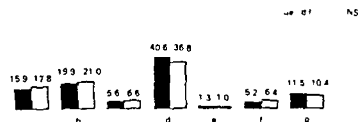


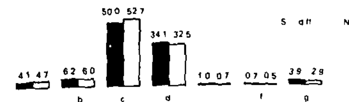
Fig 2 Frequency distribution (in percent) for *eight* questions on problems in child rearing answered by the *mothers* Separate presentation for boys and girls

and blame occurrence of discussions between parents regarding child rearing practices perceived differences between the parents and between the parents and other parents

The second group concerned the mother s problems with her four year old in the following areas toilet training sleep eating sibling contact playmate contact activity level withdrawal and obedience

The third group of questions concerned the mother action and intervention when problems occurred with the child in certain common situations like going to bed eating and playing





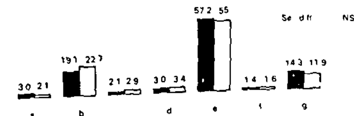






Fig 3 Frequency distribution (in percent) for five questions on actual child rearing practices in certain daily situations answered by the mothers Separate presentation for boys and girls

Table 2 *Problems of rearing reported by mothers of children with and without functionally important health problems*

	Percentage of behaviour problems reported from children											
	with health problems						without health problems					
	Neur	Som	Dent	Visual	Audit	Sum	Neur	Som	Dent	Visual	Audit	Sum
Sleep disturbance	1.6	3.2	2.1	2.4	7.5	2.6	2.7	2.3	1.9	2.7	2.5	2.7
Feeding problems	1.6	4.9	3.2	4.3	5.0	3.8	4.2	4.0	3.7	4.1	4.1	4.1
Toilet training	9.5	*0	1.1	2.4	2.6	2.4	2.0	1.9	2.3	2.0	2.2	2.2
Hyperactivity	19.7	6.6	10.6	12.6	22.5	11.5	11.4	11.3	6.3	11.5	11.4	11.6
Playmate contact	1.6	1.6	1.1	0.5	2.6	0.8	0.5	0.5	0.4	0.5	0.5	0.4
Sibling contact	7.0	3.7	2.9	3.2	0	3.1	3.4	3.1	1.7	3.5	3.5	3.5
Disobedience	3.2	4.8	4.1	2.8	2.7	3.6	3.5	3.1	2.6	3.7	3.5	3.5
Very difficult to rear 0–4 year	28.4	*8.8	9.3	9.0	17.1	11.3	9.4	8.8	6.3	9.8	10.0	9.7
Very difficult to rear just now	7.5	0	0	4.5	9.8	3.2	3.7	3.3	0.4	3.6	3.8	3.9

Statistically significant differences between children with and without health problems are indicated (see text)

Thus the analysis of the upbringing and its problems is founded on the mother's perception of their children and of the difficulties in their interaction. The relativity of the problems is then quite obvious and measures to solve these problems could be a change of the mother's attitude to her child or a treatment of a real problem in the child. However, this subjective definition of "problems" seems reasonable in a preventive program since problems perceived by parents, whether they are or not in objective terms, appears to be important to the child (15).

The mothers reported frequently use of praise (≈58%) and blame (≈58%) (Fig. 1). The total number of perceived problems was small (the median for boys was 1.7 and for girls 1.4 problems) even if the group which reported no problems at all consisted of only 25%.

Most of the reported problems concerned acting out problems, e.g. disobedience (≈40%), hyperactivity (≈35%) and quarrels with playmates (≈17%) (Fig. 2). The subgroup having siblings had a high incidence of problems in inter sibling contact. A somewhat higher incidence of problems was perceived by mothers with boys. Problems with toilet training, activity, obedience and inter sibling contact were more frequent among boys than among girls.

The answers to the third group of questions showed that mothers were quite similar in their preferred choice of action (Fig. 3). One or two among six to seven offered alternatives received a vast majority of answers. Many mothers were inclined to ignore mild forms of attention—seeking behaviour and to respond only when this behaviour was frequent or intense. One of the most

Table 3 *Problems of rearing reported by mothers of children with previously known and newly detected functionally important physical health problems*

	Percentage of behaviour problems reported from children											
	with previously known health problems						with newly detected health problems					
	Neur	Som	Dent	Visual	Audit	Sum	Neur	Som	Dent	Visual	Audit	Sum
Sleep disturbance	0	4.1	–	1.7	0	1.7	2.0	0	2.1	2.7	7.9	3.1
Feeding problems	0	6.1	–	8.2	0	6.7	2.0	0	3.2	2.7	5.3	3.2
Toilet training	30.8	0	–	3.3	0	5.0	4.0	0	1.1	2.1	2.7	2.2
Hyperactivity	0	6.3	–	10.3	50.0	8.7	24.5	7.7	10.6	13.4	21.1	12.1
Playmate contact	0	0	–	0	0	0.8	2.0	0	1.1	0.7	0	0.7
Sibling contact	0	2.4	–	5.8	0	2.8	8.7	8.3	2.9	2.2	2.9	2.9
Disobedience	0	6.1	–	1.6	0	8.3	3.9	0	4.1	3.3	5.3	3.8
Very difficult to rear 0–4 year	15.4	7.3	–	11.9	0	11.4	31.5	15.4	9.3	7.8	17.9	11.7
Very difficult to rear just now	0	0	–	4.5	0	2.3	9.3	0	0	4.7	10.3	3.2

Statistically significant differences between children with previously known and newly detected health problems are indicated (see text)

Table 4. *Use of praise and blame and perception of failure in children with and without functionally important health problems*

Percentage of children	Always praising the child for good behaviour	Always reproving the child for bad behaviour	Reporting failure in upbringing
With previously known health problems			
Neurol	53.8	61.5	8.3
Somatic	56.9	52.9	2.0
Dental	–	–	–
Visual	63.6	66.2	4.8
Audit	100.0	50.0	0
Sum	59.8	61.1	4.2
With newly detected health problems			
Neurol	60.8	68.6	2.0
Somatic	38.5	46.2	0
Dental	50.8	72.4	6.9
Visual	57.2	64.1*	6.3
Audit	63.2	55.3	0
Sum	54.6	66.3	4.7
Without health problems			
Neurol	57.8	57.8	2.9
Somatic	58.1	58.8	3.0
Dental	58.1	59.1*	2.7
Visual	57.7	57.3	2.6 *
Audit	57.6	58.2	3.0
Sum	58.4	56.4*	2.5

Statistically significant differences are indicated between children with previously known health problems and children without health problems and between children with newly detected health problems and children without health problems (see text).

preferred alternatives across situations was namely "to wait and see" and if the child insists "it gets what it desires". That means that the mother waits until the undesired behaviour is at a fairly high level of intensity.

RESULTS

A first analysis was made on the difference between children with and without functionally important physical health problems: did their mothers differ in their perception of behaviour problems?

Table 2 shows that no such difference existed when physical health problems were considered together. However, it was found that children with neurological disturbances more often had problems with their toilet training than other children (9.5% vs 2.0%, $p<0.001$) and also were considered more difficult to rear (28.4% vs 9.4%, $p<0.001$). Children with profuse caries showed more hyperactivity than other children (10.6% vs 6.3%, $p<0.05$).

From Table 3 it is also evident that these behaviour problems occurred to the same extent regardless of whether the physical deviations were detected and cared for or not. The only difference found was regarding children with known neurological defects who had more problems with toilet training than children with newly detected neurological defects (30.8% vs 4.0%, $p<0.05$). Thus whether the first hypothesis is rejected or confirmed depends on the type of health problems.

As a second hypothesis it was assumed that mothers of children with physical deviations should use more praise and less blame in their upbringing than other mothers.

As can be seen in Table 4, the use of blame as well as praise was very frequent in daily situations. However, the use of blame and rebuke was more frequent in children with physical deviations than without ($p<0.01$), and especially frequent in children with dental defects ($p<0.001$) and visual deviations ($p<0.05$). The mothers reported the same frequency of praise and blame in their upbringing of children with previously known physical defects as in children with newly detected deviations ($p<0.05$). Thus the second hypothesis is rejected.

A final hypothesis concerned the mother's perception of failure in their upbringing. As is shown in Table 4, mothers of children with visual disturbances perceived more failure than mothers of children without such deviations (6.3% vs 2.6%, $p<0.01$). The same differences were found in the groups with and without dental caries (6.9% vs 2.7%, $p<0.01$). No difference existed between newly detected and previously known disorders.

Thus again the rejection or confirmation of this hypothesis depends on the type of health problem

DISCUSSION

Most studies of behaviour problems in children with chronic diseases have dealt with children with gross handicapping disorders (2 3 27 28 35 37) implying major psychological and practical impact on the family sometimes a total reorganization of family life

In this study considerably wider definitions of health problems are used allowing also minor handicapping disorders to show their impact on the upbringing

In addition existing studies are performed on selected populations selected for either medical or social reasons To our knowledge this is the first more extensive study of the relation between physical health behavioural problems and methods of upbringing on an unselected population of preschool children

The relation between the children s physical health as defined by presence or absence of functionally important health problems and the children s behaviour as reported by their mothers was not an unequivocal one

On the whole children with health problems did not seem to cause behavioural problems for their parents more than other children and no difference was found in this respect between children with previously known (more handicapping) and newly detected (less handicapping) health problems

Punishment and rebuke as well as praise were very common as methods of upbringing whether the children had health problems or not

However and this is *important* certain groups of children were considered more troublesome and were exposed to more blame and rebuke than others Children with *neurological defects* had more problems with toilet training and were considered more difficult to rear during their first years of life Children with *dental defects* were more hyperactive Mothers of children with *dental and visual defects* used more blame in their upbringing than other mothers and consequently also perceived more failure That children with previously known neurological defects had more problems with toilet training than those with newly detected ones is probably to be explained by the fact that the former group (gross cerebral palsy hydrocephalus etc) were more seriously damaged neurologically than the latter (mostly minimal brain dysfunction) (18)

Regarding children with visual defects the interpretation could be that these children present a disturbing behaviour clumsiness and awkwardness caused by their suboptimal visual function and distorted conceptions of things around them thereby eliciting blame and rebuke from the parents

Similar reasoning is more difficult to apply in the case of dental defects Instead it is more probable that the connection between hyperactivity of the children and blame from the parents (4 29) play the greatest role i e that children with caries were more hyperactive than other children (Table 2)

Also the coexistence of caries hyperactivity and blame points towards inadequate care of the child s health and upbringing or rather an ignorance of how to promote the child s physical and mental development This view is supported also by the fact that these problems are more frequent in families with less education and poorer socio-economic background (17 24) and therefore with less knowledge of child care

The conclusion of the study is that physical health problems of milder degree do not play a great role in the development of behaviour problems However there are certain groups where it is more likely to find connections between physical health problems and behaviour problems These fami

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social obstacles to cognitive development *Genet Psychol Monogr 73* 255 1966
38 Semple J E *Hearing impaired pre school children* Charles C Thomas Springfield Ill 1970
39 Touwen B C L & Prechtl H F R *The neurological examinations of the child with minor nervous dysfunction* Little Club Clinics in Developmental Medicine no 38 Heinemann London 1970

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lies constitute risk groups with frequent parent–child conflicts They are important to identify and to advise and support in their interaction in order to reduce conflicts and relieve concern By offering education and advice about physical health as well as about child development and methods of upbringing withing the Child Health Services a more diversified and adequate climate of upbringing could perhaps be reached

REFERENCES

1 Bell R Q Stimulus control of parent or caretaker behaviour of offspring *Dev Psych 4* 63 1971
2 Bender L *Psychopathology of children with brain damage* Charles C Thomas Springfield Ill 1956
3 Bentovim A Emotional disturbances of handicapped preschool children and their families *Br Med J III* 579 1972
4 — Handicapped pre school children and their families *Br Med J III* 634 1972
5 Birch H G & Gussow J D *Disadvantaged children Health nutrition and school failure* Harcourt Brace & World Inc New York 1970
6 Buscher H *Troubles psychomoteurs chez l enfant pratique de la reeducation psycho moteur* Masson et Cie Paris 1970
7 *Child Health* Report on a European symposium WHO Copenhagen 1970
8 Decane T G A study of the mental and emotional development of the thalidomide child *In* B M Foss (ed) *Determinants of infant behaviour* IV Methuen London 1965
9 *Elections for the Riksdag 1952* Central Bureau of Statistics Stockholm 1953
10 Ely K P Henly A & Smidt G L Mother s expectations of their child s accomplishment of certain gross motor skills *Dev Med Child Neurol 14* 621 1972
11 Francis Williams J Problems of development of children with minimal brain damage *In* M Bax & R MacKeith (eds) *Minimal cerebral dysfunction* Little Club Clinics in Developmental Medicine no 10 Heinemann London 1963
12 Gewirtz J L The role of stimulation in models for child development *In* L L Dittman (ed) *Early child care* Atherton Press New York 1968
13 — On designing the functional environment of the child to facilitate behavioral development *In* L L Dittman (ed) *Early child care* Atherton Press New York 1968
14 — *Attachment and dependency* J Wiley Washington 1972
15 Haggerty R J Research issues in child health Some medical and economic issues *Pediatrics 45* 702 1970
16 Harper L V The young as a source of stimuli controlling caretaker behaviour *Dev Psych 4* 73 1971
17 Köhler F M *En pedagogisk analys av uppfostran* (with an English summary) Studentlitteratur Lund 1973
18 Kohler L Physical examination of 4-year-old children *Acta Paediatr Scand 62* 181 1973
19 Kohler L & Stigmar G Vision screening of 4-year old children *Acta Paediatr Scand 62* 17 1973
20 Kohler L & Holst H E Auditory screening of 4 year-old children *Acta Paediatr Scand 61* 555 1972
21 Köhler L & Holst K Dental health of 4 year-old children *Acta Paediatr Scand 62* 269 1973
22 — Malocclusion and sucking habits of four year-old children *Acta Paediatr Scand 62* 373 1973
23 Kohler L Fritz H & Scherstén B Bacteriuria in 4 year old children *Acta Paediatr Scand 61* 289 1972
24 Köhler L Health control of four year old children An epidemiological study of child health *Acta Paediatr Scand* Suppl *235* 1973
25 Love H D *Parental attitudes toward exceptional children* Charles C Thomas Springfield Ill 1970
26 Lowit I M Social and psychological consequences of chronic illness in children *Dev Med Child Neurol 15* 75 1973
27 Maddison D & Raphael B Social and psychological consequences of chronic disease in childhood *Lancet II* 1265 1971
28 Moor L Problèmes psychologiques des enfants et des adolescents handicapes physiques *Concours Medical 93* 5093 1971
29 Patterson G R Reprogramming the families of aggressive boys *In* C E Thorsen (ed) *Behaviour modification in education* National Assoc for the education of young children Washington 1973
30 Pless I B & Douglas J W B Chronic illness in childhood I Epidemiological and clinical characteristics *Pediatrics 47* 405 1971
31 Pond D A Is there a syndrome of brain damage in children? *Cerebral Palsy Bull 2* 296 1960
32 Prechtl H F R & Stemmer Ch J The choreiform syndrome in children *Dev Med Child Neurol 4* 119 1962
33 Prechtl H F R The mother child interaction in babies with minimal brain damage *In* B M Foss (ed) *Determinants of infant behaviour II* Methuen London 1963
34 Rheingold H L The social and socializing infant *In* D A Goslin (ed) *Handbook of socialization theory and research* Rand McNally Chicago 1969
35 Richardson S A Some social psychological consequences of handicapping *Pediatrics 32* 291 1963
36 Rutter M Tizard J & Whitmore K *Education health and behaviour* Longman London 1970
37 Shere E & Kastenbaum R Mother-child interaction in cerebral palsy environmental and psycho

Table 1 *Details of children in the trial*

Case no	Age (years)	Sex	Duration of asthma (years)	Height centile	Weight centile	Skin tests	Other atopy	Previous steroids <1 month	Chest Deformity	Hyperinflation	CXR Abnormal
1	10 0	F	9 5	8	8	+	Eczema rhinitis	−	+	+	+
2	9 9	M	9 0	5	20	+	Rhinitis	+	+	+	+
3	9 8	M	5 0	8	15	+	Rhinitis	+	+	+	−
4	14 1	M	7 0	25	40	+	Hay fever	+	+	+	−
5	10 0	M	9 5	3	25	+	Eczema	−	+	+	+
6	9 8	F	9 5	20	3	+	Eczema	−	+	+	−
7	10 5	F	7 0	15	40	+	Eczema	+	+	+	−
8	15 1	M	14 25	10	8	+	Hay fever	+	+	+	+
9	10 5	M	10 0	80	20	+	Eczema	−	+	+	+
10	14 3	M	13 0	<3	<3	+	Eczema	+	+	+	+
11	13 5	F	13 0	50	20	+	Eczema	+	+	+	+
12	13 4	M	6 5	<3	<3	+	Eczema	−	+	+	+
13	12 1	M	9 5	50	50	+	Eczema	−	+	+	+
14	10 8	M	9 0	8	10	+	Hay fever	−	+	+	+
15	11	M	9 0	12	5	+	Rhinitis	−	+	+	+
16	6 9	F	6 5	70	65	+	Eczema	+	+	+	+
17	9 1	M	8 0	15	5	+	Eczema	−	+	+	−
18	11 0	F	10 75	<3	3	+	Eczema	−	+	+	+

with continuing or previous infantile eczema All the children had unequivocal signs of chest deformity (one or more of barrel chest pigeon chest and Harrison s sulcus deformity) and their chests were hyperinflated (thoracic gas volume more than 25% above the expected mean normal for height) Fourteen had chest X ray hanges (increased bronchial wall markings together with marked hyper inflation) The majority were stunted and under weight The mean height centile for the groups was 21 weight 19 9 children were below the 10th centile for height and 9 were below the 10th centile for weight

The trial was designed on a double blind crossover basis months on active and 2 months on placebo therapy The children were randomly allocated to either active or placebo treatment during the first 2 months

The nature of the trial was fully explained to the parents and the children After initial instruction each child in haled two puffs from the aerosol either active or placebo four times a day Each puff of active aerosol contained 100 μg B17V The placebo aerosol contained inert fluohydrocarbon propellant Care was taken to explain to the patients and the parents that the aerosol was not a demand treatment that no immediate relief should be expected and that the aerosol should be used eight puffs per day throughout the trial Each ch ld was given a supply of sympathomimetic tablets (Salbutamol 2 mg) to take as necessary for asthmatic symptoms

Criteria fo imp ovement

Ch ldren were assessed by cl nical exam nation laboratory in est gations diary records and twice daily peak flow rate measurements

(a) *D ary rec d cords* Daily symptoms were scored by the child p rent grade 0 (none) to or 3 (severe) for night wheeze activity and cough as described by Connolly & Godfrey (7) The number of Salbutamol tablets taken was also recorded as were any other symptoms and any other medicines taken Disodium cromoglycate plain was con tinued if the child was established on it as were antihistamines for nasal or skin symptoms

(b) *Twice daily peak flow rates* were measured at home before the morning dose and before the evening dose of aerosol using an adult size Wright Peak Flowmeter (Airmed) The best of three puffs on each occasion was recorded

(c) *Clinical assessment* The children were seen monthly throughout the trial and enquiry was made for possible side effects of the drug Each child was weighed and measured on each visit For chest examination a simple numerical assessment of degree of wheezing was used 0=absent 1=occasional rhonchus 2=wheezy 3=tight and wheezy

(d) Adrenal function was assessed by a short tetracosactrin test at the beginning at the crossover 2 month stage and at the completion of the trial 0 25 mg tetracosactrin was injected intramuscularly after a base line cortisol collection and a further plasma cortisol was taken 30 minutes later

(e) *Respiratory Function Tests*

(I) Resistance measurements (to assess large airways ob struction)

(i) Peak Expiratory Flow Rate (PEFR) using a Wrigl t Peak Flowmeter (child size if the reading failed to reach 60 l/minute)

(ii) Forced Expiratory Volume in 0 75 seconds (FEV0 75) and Forced Vital Capacity (FVC) using a 150 l reverse plethysmograph

(iii) Airways Resistance (RAW) in a constant volume total body plethysmograph

BETAMETHASONE 17 VALERATE IN CHILDHOOD ASTHMA

A Double blind Crossover Trial in Children not Taking Other Corticosteroid Therapy

BRENT TAYLOR and A P NORMAN

From the Hospital for Sick Children Great Ormond Street London England

ABSTRACT Taylor B and Norman A P (The Hospital for Sick Children Great Ormond Street London England) Betamethasone 17 valerate aerosol in childhood asthma A double-blind crossover trial in children not taking other corticosteroid therapy Acta Paediatr Scand 64 234 1975 —A duble-blind crossover trial of Betamethasone 17 Valerate steroid aerosol compared with placebo was carried out on 18 children with severe intermittent or chronic asthma Seventeen of the 18 children showed a clinically obvious improvement on the active drug The remaining child was unable to use the inhaler properly In 14 children the response was dramatic Improvement was shown at home by a reduction in the number of asthmatic symptoms improvement in twice daily peak flow rates and by a lowered intake of sympathomimetic tablets At monthly clinic visits improvement was shown by chest examination weight gain and by detailed respiratory function tests By each of these criteria for improvement the benefit of Betamethasone 17 Valerate over the placebo was highly significant There were no side effects attributable to the therapy and no evidence as judged by short tetracosactrin tests of adrenal suppression resulting from the drug Betamethasone 17 Valerate appears to be a safe and effective therapy in children with severe asthma

KEY WORDS Asthma corticosteroid aerosols

Many paediatricians are reluctant to use oral corticosteroids in asthmatic children even severely asthmatic children because of side effects Prolonged corticosteroid therapy is liable to cause suppression of growth in children in addition to the problems which also occur in adults so treated suppression of the hypothalamo pituitary adrenal axis iatrogenic cushingism and reduced resistance to infection

It has been the policy over recent years in our Asthma Clinic to avoid steroid therapy

The recent introduction of inhaled corticosteroid preparations highly active locally but poorly absorbed systemically and reports of clinical efficiency in adults (1 4 5 10 12) encouraged us to try Betamethasone 17 Valerate (B17V) on a double blind crossover basis on a group of severely asthmatic children who were not taking oral corticosteroid therapy or receiving ACTH injections

METHODS

Eighteen children who had been attending the clinic for at least 2 years were studied They all had chronic or severe intermittent asthma Grade C or D by the criteria of McNichol & Williams (14) Eight had previously been on oral corticosteroid therapy for longer than a month but none had received more than an occasional short course (3–7 days) of oral corticosteroids or received ACTH injections during the preceding 2 years Eight were taking disodium cromoglycate and the remainder had been given a thorough trial of this drug but had discontinued it as being of no benefit Details of the patients age duration of asthma atopic status physical physiological and radiological state are shown in Table 1

They were all prick skin test positive to at least three common allergens all had other atopic symptoms twelve

Table 1 *Details of children in the trial*

Case	Age (years)	Sex	Duration of asthma (years)	Height centile	Weight centile	Skin tests	Other atopy	Previous steroids <1 month	Chest Deformity	Hyperinflation	CXR Abnormal
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6	9 8	F	9 5	70	3	+	Eczema	–	+	+	+
7	10 5	F	7 0	15	40	+	Eczema	+	+	+	–
8	15 1	M	14 5	10	8	+	Hay fever	+	+	+	+
9	10 5	M	10 0	80	20	+	Eczema	–	+	+	+
10	14 3	M	13 0	<3	<3	+	Eczema	+	+	+	+
11	13 5	F	13 0	50	70	+	Eczema	+	+	+	+
12	13 4	M	6 5	<3	<3	+	Eczema	–	+	+	+
13	1? 1	M	9 5	50	50	+	Eczema	–	+	+	+
14	10 8	M	9 0	8	10	+	Hay fever	–	+	+	+
15	11 ?	M	9 0	1?	5	+	Rhinitis	–	+	+	+
16	6 9	F	6 5	70	65	+	Eczema	+	+	+	+
17	9 1	M	8 0	15	5	+	Eczema	–	+	+	–
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with continuing or previous infantile eczema All the children had unequivocal signs of chest deformity (one or more of barrel chest pigeon chest and Harrison s sulcus deformity) and their chests were hyperinflated (thoracic gas volume more than 5 % above the expected mean normal for height) Fourteen had chest X ray changes (increased bronchial wall markings together with marked hyper inflation) The majority were stunted and under weight The mean height centile for the groups was 21 weight 19 9 children were below the 10th centile for height and 9 were below the 10th centile for weight

The trial was designed on a double blind crossover basis 2 months on active and 2 months on placebo therapy The children were randomly allocated to either active or placebo treatment during the first months

The nature of the trial was fully explained to the parents and the children After initial instruction each child inhaled two puffs from the aerosol either active or placebo four times a day Each puff of active aerosol contained 100 μg B17 V The placebo aerosol contained inert fluohydrocarbon propellant Care was taken to explain to the patients and the parents that the aerosol was not a demand treatment that no *immediate relief* should be expected and that the aerosol should be used eight puffs per day through ut the trial Each child was given a supply of sympathom meti tablets (Salbutamol mg) to take as necessary for asthmati symptoms

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(*b*) *Twice daily peak flow rates* were measured at home before the morning dose and before the evening dose of aerosol using an adult size Wright Peak Flowmeter (Airmed) The best of three puffs on each occasion was recorded

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(iii) Airways Resistance (RAW) in a constant volume total body plethysmograph

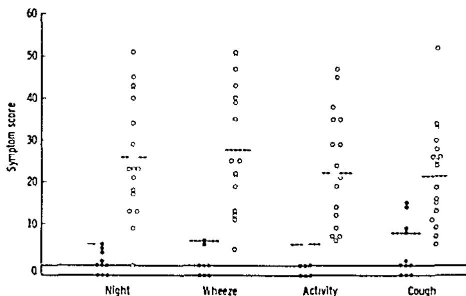


Fig 1 Individual mean symptom scores for the second month on B1 (●) and the second month on pla (O) Mean values for all 18 children

(2) Lung volumes (hyperinflation reflecting smaller peripheral airways obstruction)

(i) Function Residual Capacity (FRC) using a helium dilution technique

(ii) Thoracic Gas Volume (TGV) in the total body plethysmograph

Details of these techniques are to be published (6) The results of these measurements at each visit were expressed as a percentage of the mean normal value for London children of the same height (6) Baseline values were obtained at the beginning of the trial and once during the preceding 6 weeks

At the completion of the trial the results were assessed comparing the active treatment with the placebo period Months 1 and 2 on B17V or placebo (Months 1 and 3 and Months 2 and 4 of the trial) were assessed separately for symptom scores and home peak flow readings to avoid differences that might result from delay in the drugtaking effect or any carry over effect For the other criteria for assessment of change each complete 2 month period B17V or placebo was used for analysis

RESULTS

Seventeen of the children showed a clinically obvious improvement during the 2 months on B17V compared with the other 2 months on placebo The remaining child (Case No 6) was unable to synchronise her breathing with the release of the aerosol despite physiotherapy tuition and so was unable to inhale the drug She showed no benefit and was in fact worse by some criteria while on the active drug

(a) *Symptom scores twice daily peak flow rates and Salbutamol intake*

The mean score for each child during the second month on B17V compared with the second month on placebo for night symptoms wheez activity and cough is shown in Fig 1 All b Case 6 were better on B17V A similar i provement on B17V compared with place was shown on morning and evening peak fl readings (Fig 2) All but Case 6 improved children reached mean levels more than tw their mean placebo period readings

The mean peak flow readings mean total symptom score and mean Salbutamol consumption for all the children during the second month on B17V and the second month on placebo is shown in Table 2 In each case the children were significantly better on B17V

(b) *Clinical changes*

The mean chest examination score for all the children during the 2 months on B17V (total possible 6 0) compared with the 2 months on placebo is shown in Table 3 together with the average height and weight gain for each of the two periods The children as a whole were significantly better on chest examination and gained significantly more weight on B17V There was no significant difference in height gain on the two regimes

(c) *Respiratory Function Tests*

The children as a whole showed significant improvement in FEV 0 75 FVC PEFR RAW and TGV measurements on B17V compared with the values obtained during the placebo

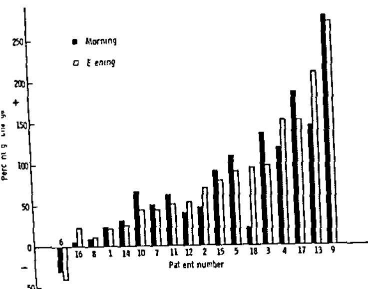


Fig 2 Morning and evening peak expiratory flow rates showing the mean percentage change during the second month on B17V compared with the mean values obtained during the second month on placebo

period The results on B17V for these studies were also significantly better than those obtained during the baseline period before the trial began There was no significant difference between the mean placebo reading and the mean baseline values nor between any of the FRC results (Fig 3)

(d) Effect of the length of therapy

Although the children as a whole were significantly better during both months on B17V compared with the 2 months on placebo the difference was most marked during the second month and a few children were less well during their first month on B17V than they were during the first month on placebo The mean values were better during the second month on B17V and worse during the second month of placebo in terms of peak flow readings and asthmatic symptoms compared with the first months but the differences were not statistically significant (Table 4) It was not possible to determine whether the less marked improvement during the first month was due to a delay in the drug taking effect or to a carry over effect resulting in the values during the first placebo month being abnormally good in those children going from B17V to placebo during the trial There was no significant difference in symptoms or twice daily peak flow rates between these children during the first month on placebo and the first month values in those children who began the trial with placebo therapy

Table 2 *Mean values for the 18 children for morning and afternoon peak flow rates total symptom scores and Salbutamol intake during the second month on B17V and the second month on placebo*

	B17V	Placebo	p
M rning PEFR l/m n	04 1	1 6 8	<0 001
E en ng PEFR l/m n	19 9	136 1	<0 001
T tal ympt m core	5 0	98 9	<0 005*
S lbutamol intake (mg tablet)	3 8	65 8	<0 005*

Pa red T te t
R nk Sum te t

(e) State of children s asthma

Two children who showed marginal benefit from B17V during the trial appear to have been entering a remission period of their asthma and have remained well since the trial without steroids on limited therapy Another girl although improved was still severely asthmatic on B17V Excluding the girl who had technical difficulties using the aerosol the remaining

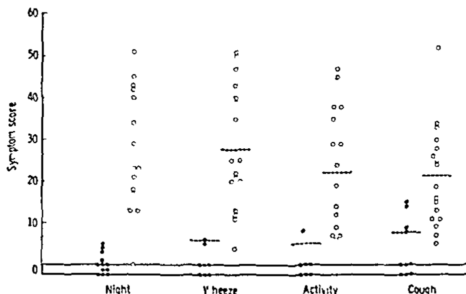


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(a) Symptom scores twice daily peak flow rates and Salbutamol intake

The mean score for each child during the second month on B17V compared with the second month on placebo for night symptoms wheeze activity and cough is shown in Fig 1 All but Case 6 were better on B17V A similar improvement on B17V compared with placebo was shown on morning and evening peak flow readings (Fig 2) All but Case 6 improved 6 children reached mean levels more than twice their mean placebo period readings

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(c) Respiratory Function Tests

The children as a whole showed significant improvement in FEV 0 75 FVC PEFR RAW and TGV measurements on B17V compared with the values obtained during the placebo

results so effective was B17V in controlling wheeze

We believe that an increase in height and weight gain is a very good guide to progress in childhood asthma and have previously shown an improvement in these indices following hyposensitisation therapy (16) The weight increases on B17V reflected improvement in the general state with increased appetite following remission of the asthma and not fluid retention due to the steroids because none of the children who went from active to placebo therapy during the trial showed a sudden loss of weight and vice versa It was not unexpected that the height gain would show no difference during the short duration of the study

A highly significant difference between B17V and placebo therapy was found on respiratory function testing We used FEV 0 75 as it has been shown to be a more sensitive and reliable index of airways obstruction than the FEV 1 0 in childhood (6) The only respiratory function test not showing improvement in our study was the functional residual capacity This may be due to air trapping which is frequently found in children with severe asthma The total air in the lungs including trapped air is measured by the TGV technique FRC only measures air in communication with the atmosphere In support of this explanation is the finding of a reduction in the difference between the FRC and the TGV on B17V (Fig 3) Other workers have considered interval lung function testing of little benefit in childhood asthma (3 15) Indeed Godfrey & Konig state that such random measurements are useless in assessing results of drugs in trials (11) It is possible that these workers have included in their studies children with mild asthma where the results of respiratory function tests are often normal apart from during an attack Children with less easily reversible asthma tend to show continuing physiological abnormalities Certainly we have demonstrated useful changes in respiratory function with effective therapy in these severely asthmatic children No side effects from the drug were discovered during this trial There is a possibility of reducing local resistance with topical steroids and recently a high incidence of candida infection of the throat has been reported in patients on long term therapy (13) We did not swab the throats of the children in our study but none complained of sore throat Another possible hazard after the local administration of corticosteroids is the development of an atrophy of the mucous membranes or other respiratory tissues There have been no reports of such atrophy in either the experimental or clinical situation but the long term safety of such preparations is unproven

Apart from safety the continuing effectiveness of steroid aerosol therapy over prolonged periods is obviously of great importance Other investigators reporting the continuing use of Beclomethasone dipropionate have intimated that that drug may lose its effectiveness in some children after a period (8 11) This has not been our experience with the continuing use of B17V for up to eleven months indeed rather the reverse A surprisingly low dose appears to remain effective in largely abolishing asthmatic symptoms Fourteen of the eighteen children in the trial remain on B17V Eight are well controlled on one puff twice a day and only one child is taking eight puffs a day

ACKNOWLEDGMENTS

We would like to record our thanks to Miss A Y Chamberlain for help with this study particularly the statistical analysis of the results and to Glaxo Laboratories Ltd for equipment and materials

REFERENCES

1 Brown H M Storey G & George W H S Beclomethasone dipropionate aerosol and prednisolone in reversible airways obstruction *Br Med J III* 314 1972

2 Brown H M & Storey G Beclomethasone dipropionate steroid aerosol in treatment of perennial allergic asthma in children *Br Med J III* 161 1973

3 Chai H Purcell K Brady K & Falliers C J Therapeutic and investigational evaluation of asthmatic children *J Allergy 41* 23 1968

4 Choo Kang Y F J Cooper E J Tribe A E & Grant I W B Beclomethasone dipropionate by inha

Table 3 *Mean values for the 18 children for chest examination score weight and height gain during the 2 months on B17V and the 2 months on placebo*

	B17V	Placebo	*p*
Chest examination score	0 55	3 44	<0 005[a]
Weight gain (kg)	1 40	0 37	<0 001[b]
Height gain (cm)	0 56	0 59	N S[b]

[a] Rank Sum test
[b] Student *t* tests

fourteen children showed a dramatic improvement on B17V

(f) Side effects tetracosactrin tests

There were no side effects detected attributable to the therapy and there was no evidence of adrenal suppression resulting from the drug

DISCUSSION

B17V appears to be a safe and effective therapy in children with severe asthma

Frear et al (9) have recently published their results of B17V in childhood asthma our results show comparable benefit from the drug

Previous reports of inhaled steroid aerosol in childhood asthma (2 8 11) were carried out almost wholely on children already receiving other steroid therapy These investigators considered it unethical to give placebo alone to steroid dependent children but as a result it was impossible to demonstrate an unequivocal effect from this type of drug because similar drugs were given at the same time

Table 4 *Mean Peak Respiratory flow rates and symptom scores for the 18 children during the first and second months on B17V and placebo*

	B17V		Placebo	
	Month 1	Month 2	Month 1	Month 2
Morning PEFR	180 5	204 1	136 6	1[illegible]6 8
Evening PEFR	206 6	219 9	146 3	136 1
Night symptom score	7 1	5 1	23 [illegible]	25 8
Wheeze score	8 4	5 7	25 2	[illegible]7 6
Activity score	8 3	5 0	22 4	[illegible]1
Cough score	5 4	7 7	17 3	[illegible]1 3
Total symptom score	29 2	[illegible]3 0	88 1	96 8

We have demonstrated benefit during the period on this active drug compared with the period on the placebo by each of the criteria taken for improvement

Effective therapy is well known to lead to reduced drug usage and improvement in symptom score and twice daily peak flow rates The other criteria used to assess improvement in this study are not so widely recognised The improvement in chest examination score is perhaps rather less impressive than it appears A simple plus–wheeze minus–no wheeze scoring system would probably have given similar

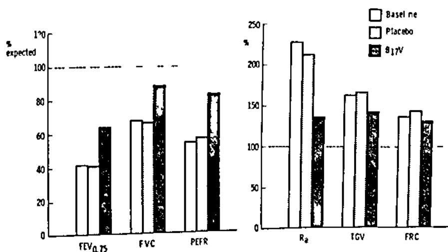


Fig 3 Respiratory Function Tests showing the mean values expressed as a percentage of the mean normal values during the 2 months on B17V the 2 months on placebo and the baseline period

Acta Pædiatr Scand 64 241–247 1975

AN EPIDEMIOLOGICAL STUDY OF CHILD HEALTH AND NUTRITION IN A NORTHERN SWEDISH COUNTY

VII A Comparative Study of General and Dental Health Food Habits and Socio economic Conditions in 4 year old Children

GÖSTA SAMUELSON HANS K SON BLOMQUIST CLAES-GÖRAN CROSSNER ANNA KARIN HOLM and HANS GRAHNÉN

From the Departments of Paediatrics and Pedodontics University of Umeå Umea Sweden

ABSTRACT Samuelson G Blomquist H K son Crossner C G Holm A K and Grahnen H (Departments of Paediatrics and Pedodontics University of Umeå Umeå Sweden) An epidemiological study of child health and nutrition in a northern Swedish county VII A comparative study of general and dental health food habits and socio-economic conditions in 4-year-old children Acta Paediatr Scand 64 241 1975.—A study of the general and dental health and the food habits of randomly selected 4-year-old Swedish urban children was performed The results were compared with the findings of an investigation carried out four years earlier in the same area In comparison with the earlier study no significant differences were found in haemoglobin values packed red cell volume microsedimentation rate and anthropometric measurements The food habits had altered A reduction in the frequency of between meal consumption particularly of sweets and soft drinks as well as a reduction of the frequency of meat fish and egg consumption was found The children had an increased sandwich and milk consumption The caries frequency was markedly reduced which might be explained by the decreased between meal consumption and an increased consumption of fluoride tablets The food habits and the caries situation were generally influenced by the parents socio-economic conditions especially their educational level

KEY WORDS anthropometrical measurements caries and gingivitis food habits and socio-economic conditions

In 1967 a nutritional survey (9) as well as a general and oral health study (10 11 13) was performed in the northern part of Sweden in one urban and two rural areas The relationships between general and oral health food habits and socio-economic conditions were analysed (12) Three age groups 4 8 and 13 year-old children were investigated The 4 year olds were entirely from the city of Umeå

In the present study another group of 4 year-old children living in the city of Umeå were examined four years after the former investigation The main purpose was to compare the general health caries frequencies gingival state and food habits in 1971 with those of 1967 and to investigate by means of multiple regression analyses the connection between dental health food habits and socio-economic conditions

MATERIAL

The material of the 1971 study consisted of 187 4 year old children (86 boys and 101 girls) from the city of Umeå The children were randomly selected (?) from the official population register of the real urban part of

lation in the treatment of airways obstruction *Br J Dis Chest* 66 101 1972
5 Clark T J H Effect of beclomethasone dipropionate delivered by aerosol in patients with asthma *Lancet* I 1361 1972
6 Cogswell J J Hull D Milner A D Norman A P & Taylor B Normal lung function in childhood Part 1 Forced expiratory volumes Part 2 Resistance measurements Part 3 Lung volumes Thoracic gas volume and functional residual capacity *Br J Dis Chest* in press
7 Connolly N M & Godfrey S Assessment of the child with asthma *J Asthma Res* 8 31 1970
8 Dickson W Hall C F Ellis M & Horrocks R M Beclomethasone dipropionate aerosol in childhood asthma *Arch Dis Child* 48 671 1973
9 Frears J F Wilson L C & Friedman M Betamethasone 17 valerate by aerosol in childhood asthma *Arch Dis Child* 48 856 1973
10 Gaddie J Petrie G R Reid I W Sinclair D J M Skinner C S Palmer K N V Aerosol beclomethasone dipropionate in chronic bronchial asthma *Lancet* I 691 1973
11 Godfrey S & Konig P Beclomethasone aerosol in childhood asthma *Arch Dis Child* 48 665 1973
12 Lal S Harris D M Bhalla K K Singal S N & Butler A G Comparison of beclomethasone dipropionate aerosol and prednisolone in reversible airways obstruction *Br Med J* III 314 1972
13 McAllen M K Kochanowski S J & Shaw K M Steroid aerosols in asthma An assessment of betamethasone valerate and a 12 month study of patients on maintenance treatment *Br Med J* I 171 1974
14 McNicol K N & Williams H B Spectrum of asthma in children I Clinical and physiological components *Br Med J* IV 7 1973
15 Silverman M Connolly N M Balfour Lynn L & Godfrey S Long term trial of disodium cromoglycate and isoprenaline in children with asthma *Br Med J* III 378 1972
16 Taylor B Sanders S S Norman A P A double blind controlled trial of house mite fortified house dust vaccine in childhood asthma *Clin Allergy* 4 35 1974

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AN EPIDEMIOLOGICAL STUDY OF CHILD HEALTH AND NUTRITION IN A NORTHERN SWEDISH COUNTY

VII A Comparative Study of General and Dental Health Food Habits and Socio economic Conditions in 4 year old Children

GÖSTA SAMUELSON HANS K SON BLOMQUIST CLAES-GÖRAN CROSSNER
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ABSTRACT Samuelson G Blomquist H K son Crossner C G Holm A K and Grahnen H (Departments of Paediatrics and Pedodontics University of Umeå Umeå Sweden) An epidemiological study of child health and nutrition in a northern Swedish county VII A comparative study of general and dental health food habits and socio-economic conditions in 4-year-old children Acta Paediatr Scand 64 241 1975.—A study of the general and dental health and the food habits of randomly selected 4-year-old Swedish urban children was performed The results were compared with the findings of an investigation carried out four years earlier in the same area In comparison with the earlier study no significant differences were found in haemoglobin values packed red cell volume microsedimentation rate and anthropometric measurements The food habits had altered A reduction in the frequency of between meal consumption particularly of sweets and soft drinks as well as a reduction of the frequency of meat fish and egg consumption was found The children had an increased sandwich and milk consumption The caries frequency was markedly reduced which might be explained by the decreased between meal consumption and an increased consumption of fluoride tablets The food habits and the caries situation were generally influenced by the parents socio-economic conditions especially their educational level

KEY WORDS anthropometrical measurements caries and gingivitis food habits and socio-economic conditions

In 1967 a nutritional survey (9) as well as a general and oral health study (10 11 13) was performed in the northern part of Sweden in one urban and two rural areas The relationships between general and oral health food habits and socio-economic conditions were analysed (12) Three age groups 4 8 and 13 year-old children were investigated The 4 year olds were entirely from the city of Umeå

In the present study another group of 4 year-old children living in the city of Umeå were examined four years after the former investigation The main purpose was to compare the general health caries frequencies gingival state and food habits in 1971 with those of 1967 and to investigate by means of multiple regression analyses the connection between dental health food habits and socio-economic conditions

MATERIAL

The material of the 1971 study consisted of 187 4 year old children (86 boys and 101 girls) from the city of Umeå The children were randomly selected (2) from the official population register of the real urban part of

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7 Connolly N M & Godfrey S Assessment of the child with asthma *J Asthma Res* 8 31 1970
8 Dickson W Hall C L Ellis M & Horrocks R M Beclomethasone dipropionate aerosol in childhood asthma *Arch Dis Child 48* 671 1973
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In 1967 a nutritional survey (9) as well as a general and oral health study (10 11 13) was performed in the northern part of Sweden in one urban and two rural areas The relationships between general and oral health food habits and socio economic conditions were analysed (12) Three age groups 4 8 and 13 year-old children were investigated The 4 year olds were entirely from the city of Umeå

In the present study another group of 4 year-old children living in the city of Umeå were examined four years after the former investigation The main purpose was to compare the general health caries frequencies gingival state and food habits in 1971 with those of 1967 and to investigate by means of multiple regression analyses the connection between dental health food habits and socio-economic conditions

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Table 1 *Mean values and standard deviations (S D) of the skinfold thickness (mm) and of the circumference of the upper arm (cm) and the circumference of the upper arm muscle (cm)*

		Triceps skin fold			Subscapular skinfold			The circum ference of the upper arm			The circum ference of the upper arm muscle	
	n	Means	S D	*n*	Means	S D	*n*	Means	S D	*n*	Means	S D
Boys	84	8 9	1 7	85	5 3	1 4	86	16 9	1 1	84	14 1	1 1
Girls	97	9 9	1 6	97	6 2	2 0	99	17 1	1 3	97	14 0	1 1
Total	181	9 4	1 7	18[illegible]	5 8	1 8	185	17 0	1 [illegible]	181	14 1	1 1

Anthropometrical investigation

Height and weight In comparison with the examination performed in 1967 (10) there was no significant difference in height either for the boys or the girls

Subcutaneous fat (Table 1) The girls had a somewhat greater subscapular and triceps skinfold ($p<0.05$) than in the earlier examination (10)

Upper arm circumference (Table 1) No significant sex differences in either upper arm or upper arm muscle circumference were found

Odontological investigation

The dental status is described in detail by Holm (3) In comparison with the earlier study (11) the caries frequency was reduced (Tables 2 and 3) The number of caries free children was increased from 17% to 33% The difference is significant ($p<0.001$) The consumption of fluoride tablets (Table 4) was increased ($p<0.001$) There were no significant differences in the tooth brushing frequency

Food consumption frequencies—comparison 1967 to 1971

The consumption frequencies of vegetables and potatoes were similar in 1971 and in 1967 (Figs 1 *a* and *c*) The consumption of fruits and berries was for the majority of children reduced from twice to once a day (Fig 1 *b*) The milk and cheese consumption was more frequent than in 1967 (Fig 1 *d*)—15% of the children consumed milk four times a day The consumption of full milk was the same as that of skim milk In 1967 80% of the children consumed something from the group meat fish and eggs several times a day In 1971 the comparable figures was 5% The majority of the children 83% had something from this food group once a day (Fig 1 *e*) The cereal consumption was nearly the same (Fig 1 *f*) The dietary fat intake was more frequent in 1971 due to higher consumption of sandwiches (Fig 1 *g*) The between meal eating of sweets soft drinks and buns and cakes more than once a day was reduced (Fig 1 *h*) from 60% in 1967 to 20% in 1971

Table 2 *Means and standard deviations (S D) of deft values (decayed extracted and filled teeth) in 1967 and 1971*

		1967			1971		
	n	Means	S D	*n*	Means	S D	Difference
Boys	98	5 [illegible]9	4 0	86	3 33	3 30	1 96
Girls	98	5 40	4 89	101	3 15	3.59	2 25
Total	196	5 31	4 47	187	3 [illegible]3	3 47	2 08

the city of Umeå in the same way as in the earlier study (9) The 1967 study comprised 198 4 year-olds (99 boys and 99 girls)

The mean and median age of the children investigated in 1967 and 1971 was 4 years and 5 months and 4 years and 6 months respectively

METHODS

The children were examined by one paediatrician and one dentist in 1967 and two others in 1971 The investigation was carried out during the same time of the year as in the earlier study The *physical examination* the *anthropometrical measurements* (weight height triceps and subscapular skinfolds) the *haematological examinations* (haemoglobin packed red cell volume blood smear examination) the *determination of the microsedimentation rate* (ESR) were performed as previously (10 13) In addition in the present study the circumference of the left upper arm was measured using a measuring tape and recorded to within 0 1 cm By the aid of this measurement the muscular circumference of the upper arm was calculated (5) In the ESR and the haematological examination 13 and 15 children respectively were omitted due to lack of cooperation and for technical reasons

Odontological examination The children were examined clinically and roentgenologically in the same way as in the earlier study (11) However dental plaque was not recorded as one could expect more children to have brushed their teeth more thoroughly than usual before the examination Satisfactory radiographs could not be obtained from 15% of the children (3)

Method of dietary investigation The frequency of child s consumption of different foods was recorded by means of a food habit history The interview was *performed personally in the child s home with the* mother in attendance by one of the trained interviewers from the previous study The same questionnaire was used as in 1967 and registered the frequency of consumption as never once every other month twice a month once a week several times a week once a day 2–3 times a day and 4 or more times a day (9)

Socio economic investigation In addition to the dietary interview socio economic data described below were gathered

Variables used in the stepwise multiple regression analyses

The same variables were used as in the previous study (12) In addition upper arm circumference as well as two variables *of between meal eating* described on pp 243 and 244 respectively were added

Medical variables Haemoglobin (Hb) packed red cell volume (PCV) erythrocyte microsedimentation rate (ESR) height weight skinfold measurements (triceps and subscapular skinfold) number of colds per year and upper arm circumference

Odontological variables (a) caries indices decayed extracted filled teeth and surfaces (deft and defs) *(b) gingival index (GI) according to Loe & Silness (8) (c)* frequency of tooth brushing times per day

Food frequency consumption variables

Vegetables fruits berries meat fish eggs sausage porridge and between meal consumption of sweets buns and cakes soft drinks and fruits Variables with approximately the same frequency for most of the children e g milk and potatoes were excluded The analyses are described in Table 5 In analyses 5 and 6 two variables of between meal eating were constructed besides the groups sweets and fruits namely (*a*) soft drinks and juices with or without buns and cakes designated snacks I (*b*) chips pop-corn peanuts and cheese-doodles designated snacks II

Socio economic and demographic variables Mother s age parents education (number of years of education and types of school attended) family s total income per capita including social security support number of persons per room *number of children* and the child s age in months

Statistical methods

Student s *t* test Chi square analyses and multiple regression analyses were used (1 14) The regression analyses is described elsewhere *in detail by Samuelson et al* (12) The significance levels are * ($p<0.05$) ($p<0.01$) ($p<0.001$) where p is the probability of an erroneously rejected null hypothesis

RESULTS

Medical investigation

The general health and the clinical nutritional status were good Upper respiratory infections of minor significance *were clinically* observed in about 12% Thirty % had ESR <20 mm The corresponding figure from the study of 1967 was 38%

Haematological examination

The mean values of haemoglobin packed red cell volume and mean corpuscular haemoglobin concentration are slightly higher than the values of Samuelson & Sjolin (13) No case of anaemia and no abnormalities in the blood smears were found 60 per cent of the children with Hb and/or PCV values at or below the mean–2 SD had an ESR higher than 20 mm compared with 21% in the total material The comparable figures in the study of 1967 were 48 and 20% respectively

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Table 5 *Analyses between different groups of variables*

Analy	Regressands	Regressors
	Medical variables	Food variables
	Medical variables	Socio-economic variables
	Food variables	Socio economic variables
	Medical variables	Food variables+ socio-economic variables
	Medical variables	Four groups of between meal eating
	Four groups of between meal eating	Socio-economic variables
	Odontological variables	Medical food and socio-economic variables and tooth brushing

ere positively correlated to age and subcutaneous fat to sex

A *comparison* between the present results and the results from the study of 1967 shows that the educational level of the parents had on an average the same influence on the children s frequency consumption of different foods

DISCUSSION

The general health situation was good and the findings are in agreement with the study *of 1967 (10)* and in accord with a recent study in southern Sweden by Kohler (6) The haematological values and *the incidence* of increased ESR in the total material were approximately the same as *in the earlier* study (13) Further in the present study infections mainly upper respiratory infections appeared to have an influence on the Hb and/of PCV values The higher frequency of ESR-elevation in the group of subnormal Hb-values in comparison with the rest of the material underline the importance of infections in causing low Hb values during childhood

No certain differences were found between the children of 1967 and 1971 in the anthropometrical values of height and weight Only a slight increase in the skinfold measurements was found The food frequency *consumption study did not include* data of the nutrient content of the diet For that reason *no conclusion can be made of any dietary* influence on the skinfold measurements or *on the muscular circumference*

In comparison with the earlier study differences were found in food *habits Especially* the reduced between meal eating is of interest The reasons may be multifactorial a more active information concerning food habits in the Child Health Centres and mass media has probably been of importance The

Table 6 *Results of regression analyses 3 6 and 7*

Analyses	Regressands	Regressors entering at a significance level of $p<0.01$	β-coefficients[a]	R^2 in %
3	*Porridge*	*Mother s education*	−0 [illegible]	7 49
	Berries	Mother s education	0 3[illegible]	5 01
	Vegetables	Father s education	0 [illegible]0	4 04
	Sweets	*Mother s education*	−0 29	8 34
	Soft drinks	Mother s education	−0 [illegible]5	6 [illegible]8
6	Sweets	Mother s education	−0 29	8 34
	Snacks I	Mother s education	−0 [illegible]8	8 04
	Snacks II	Mother s education	−0 25	6 3[illegible]
7	deft	*Father s education*	−0 3	
		Sweets	0 [illegible]9	1 79
	defs	Father s education	−0 [illegible]6	
		Sweets	0 74	16 13
	GI	Soft drinks	0 [illegible]0	4 00

[a] The β-coefficient is a coefficient of regression which is independent of the particular units used for the variables Therefore the β-coefficient is a suitable tool for the comparison of the relative importance of the regressors R^2 in per cent shows the amount of the variation of the regressand explained by the regressors

Table 3 *Means and standard deviations (S D) of defs values (decayed extracted and filled surfaces) in 1967 and 1971*

	1967			1971			
	n	Means	S D	*n*	Means	S D	Difference
Boys	98	7 51	7 11	86	4 53	5 55	2 98**
Girls	98	8 04	8 70	101	4 52	6 31	3 52**
Total	196	7 78	7 93	187	4 53	5 99	3 25

Regression analyses

The educational level of the parents was consistently of importance (Table 6) A positive correlation was found between educational level and the consumption frequency of berries and vegetables a negative correlation to the frequency of consumption of porridge sweets and soft drinks (Analysis 3) Analysis 6 showed that the children of parents with a higher educational level had a decreased consumption not only of sweets soft drinks with buns and cakes (snacks I) but also a decreased consumption of chips pop-corn peanuts and cheese doodles (snacks II) Analysis 7 showed a negative correlation between caries indices and the level of the parents education A more frequent consumption of sweets increased the caries indices *An increased consumption of* soft drinks increased the gingival index Further analyses confirming these results are presented by Holm et al (4) In the analyses 1 2 4 and 5 (Table 5) only a number of self evident correlations were obtained as in the earlier study namely that height and weight

Table 4 *Consumption of fluoride tablets in 1967 and 1971 percentages within parentheses*

No of years	No of children 1967	1971
3–4	10 (5)	48 (26)
1–2	12 (6)	68 (36)
0	174 (89)	71 (38)

$\chi^2(2)=65\ 11$***

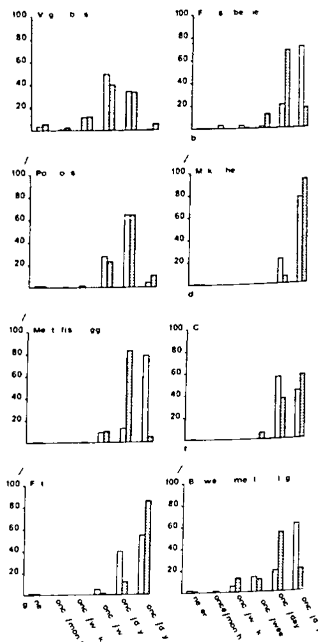


Fig 1 a–h Food frequency consumption Open bars visualize the 1967 investigation and stippled bars the results from the present study

Table 5 *Analyses between different groups of variables*

Analyses	Regressands	Regressors
[illegible]	Medical variables	Food variables
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were positively correlated to age and sub cutaneous fat to sex

A *comparison* between the present re sults and the results from the study of 1967 shows that the educational level of the par ents had on an average the same influence on the children s frequency consumption of different foods

DISCUSSION

The general health situation was good and the findings are in agreement with the study of 1967 (10) and in accord with a recent study in southern Sweden by Kohler (6) The haematological values and the incidence of increased ESR in the total material were approximately the same as in the earlier study (13) Further in the present study infections mainly upper respiratory infec tions appeared to have an influence on the Hb and/of PCV values The higher frequen cy of ESR elevation in the group of sub normal Hb values in comparison with the rest of the material underline the importance of infections in causing low Hb-values dur ing childhood

No certain differences were found be tween the children of 1967 and 1971 in the anthropometrical values of height and weight Only a slight increase in the skinfold meas urements was found The food frequency consumption study did not include data of the nutrient content of the diet For that rea son no conclusion can be made of any die tary influence on the skinfold measurements or on the muscular circumference

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Table 6 *Results of regression analyses 3 6 and 7*

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	Vegetables	Father s education	0 0	4 04
	Sweets	Mother s education	−0 ?9	8 34
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	Snacks I	Mother s education	−0 ?8	8 04
	Snacks II	Mother s education	−0 25	6 3?
7	deft	Father s education	−0 3	
		Sweets	0 ?9	?1 79
	defs	Father s education	−0 6	
		Sweets	0 4 *	16 13
	GI	Soft drinks	0 ?0	4 00

The β-coefficient is a coefficient of regression which is independent of the particular units used for the variables Therefore the β-coeffi ient is a suitable tool for the comparison of the relative importance of the regressors R in per cent shows the amount of the variation of the regressand explained by the regressors

Table 3 *Means and standard deviations (S D) of defs values (decayed extracted and filled surfaces) in 1967 and 1971*

	1967			1971			
	n	Means	S D	*n*	Means	S D	Difference
Boys	98	7 51	7 11	86	4 53	5 55	2 98*
Girls	98	8 04	8 70	101	4 52	6 31	3 52**
Total	196	7 78	7 93	187	4 53	5 99	3 25

Regression analyses

The educational level of the parents was consistently of importance (Table 6) A positive correlation was found between educational level and the consumption frequency of berries and vegetables a negative correlation to the frequency of consumption of porridge sweets and soft drinks (Analysis 3) Analysis 6 showed that the children of parents with a higher educational level had a decreased consumption not only of sweets soft drinks with buns and cakes (snacks I) but also a decreased consumption of chips pop corn peanuts and cheese doodles (snacks II) Analysis 7 showed a negative correlation between caries indices and the level of the parents education A more frequent consumption of sweets increased the caries indices An increased consumption of soft drinks increased the gingival index Further analyses confirming these results are presented by Holm et al (4) In the analyses 1 2 4 and 5 (Table 5) only a number of self evident correlations were obtained as in the earlier study namely that height and weight

Table 4 *Consumption of fluoride tablets in 1967 and 1971 percentages within parentheses*

	No of children	
No of years	1967	1971
3–4	10 (5)	48 (26)
1–2	12 (6)	68 (36)
0	174 (89)	71 (38)

$\chi^2(2)=65\ 11$ **

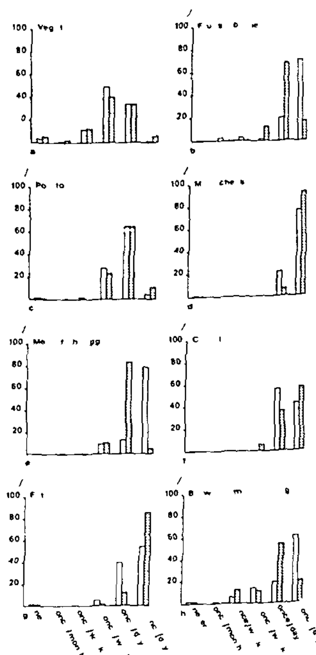


Fig 1 a–h Food frequency consumption Open bars visualize the 1967 investigation and stippled bars the results from the present study

9 Samuelson G An epidemiological study of child health and nutrition in a northern Swedish county I Food consumption survey *Acta Paediatr Scand* 60 Suppl *214* 1971

0 — An epidemiological study of child health and nutrition in a northern Swedish county III Medical and anthropometrical examinations *Acta Paediatr Scand 60* 653 1971

1 Samuelson G Grahnén H & Lindstrom G An epidemiological study of child health and nutrition in a northern Swedish county V Oral health studies *Odontol Rev 22* 189 1971

2 Samuelson G Grahnen H & Arvidsson E An epidemiological study of child health and nutrition in a northern Swedish county VI Relationship between general and oral health food habits and socio-economic conditions *Am J Clin Nutr 24* 1361 1971

13 Samuelson G & Sjolin S An epidemiological study of child health and nutrition in a northern Swedish county IV Haematological investigations especially in regard to iron deficiency anaemia *Acta Paediatr Scand 61* 63 1972

14 Snedecor G W & Cochran W G *Statistical methods* Ames Iowa 1971 6th ed

15 Weiss R L & Trithart A H Between meal eating habits and dental caries experience in preschool children *Am J Public Health 50* 1097 1960

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reduction in meat fish and egg consumption may be due to the elevated prices but is relatively small and probably of little importance The finding of a more frequent consumption of sandwiches may be an expression of a changing food habit pattern in between meal consumption and/or in ordinary meals

The decreased caries indices and the increased number of caries free children might to a considerable part be explained by the caries prophylactic program started at the Child Health Centres in 1968 where information is given concerning food habits and oral hygien Fluoride tablets were prescribed as the fluoride content of the drinking water in Umeå was very low less than 0 1 mg/l Thus the marked decrease in the caries frequency can be explained by a decrease in between meal consumption of sweets soft drinks buns and cakes and of an increase in the consumption of fluoride tablets

The results of the multiple regression analyses are of interest in the matter of information about good food habits The food frequency consumption variables probably apply to only a short period whereas the medical variables except ESR reflect a longer period This may explain that no certain correlation was found between food frequency consumption and general health On the other hand socio economic conditions particularly the parents educational level have an influence on the food habits The main results are in general the same as in the earlier study (12) A new finding is that the consumption between meals of superfluous calories like chips peanuts and cheese doodles was also negatively correlated to the parents educational level Parents with lower educational level more often seem to give their children such kinds of foodstuffs

The educational level of the parents affected the children s caries situation This is in accord with the earlier study (12) and with another Swedish study by Kohler & Holst (7) Frequent consumption of sweets between meals increased the caries indices which is a previously well known fact not only in adults and school-children but also in pre school children (15) Frequent consumption of soft drinks with or without buns and cakes increased the gingival index This type of highly sugar containing between meal eating may give a heavy accumulation of dental plaque which is known to induce a gingival inflammation

The present study emphasizes that socio economic factors especially education determine food habits It is important to be aware that the pattern of consumption is dynamic Therefore in order to be able to give dietary advice it is necessary to continually evaluate the changing food habits in the society by means of repeated investigations

ACKNOWLEDGEMENTS

Our thanks are due to Erik Arvidsson fil kand Institute of Mathematics and Statistics University of Umeå for help with the statistical analyses and to Hans Wallberg fil kand for help with the computer analyses at the Computer Centre University of Umeå

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REFERENCES

1 Cooley W W & Lohnes P R *Multivariate data analysis* John Wiley & Sons Inc New York 1971
2 Holm A K & Arvidsson S Oral health in pre school Swedish children I Three year-old children *Odontol Rev* *25* 81 1974
3 Holm A K Oral health in four year-old Swedish children *Comm Dent Oral Epidemiol* In press
4 Holm A K Blomquist H K son Crossner C G Grahnen H & Samuelson G A comparative study of oral health as related to general health food habits and socioeconomic conditions of four year-old Swedish children *Comm Dent Oral Epidemiol 3* 1975 In press
5 Jelliffe D B *The assessment of the nutritional status of the community* WHO Geneva 1966
6 Kohler L Health control of four year old children An epidemiological study of child health *Acta Paediatr Scand 62* Suppl 235 1973
7 Kohler L & Holst K Dental health of four year old children *Acta Paediatr Scand 62* 279 1973
8 Loe H & Silness J Periodontal disease in pregnancy I Prevalence and severity *Acta Odontol Scand 21* 533 1963

9 Samuelson G An epidemiological study of child health and nutrition in a northern Swedish county I Food consumption survey *Acta Paediatr Scand 60* Suppl *214* 1971

10 — An epidemiological study of child health and nutrition in a northern Swedish county III Medical and anthropometrical examinations *Acta Paediatr Scand 60* 653 1971

11 Samuelson G Grahnen H & Lindstrom G An epidemiological study of child health and nutrition in a northern Swedish county V Oral health studies *Odontol Rev 22* 189 1971

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ABSENCE OF INSULIN RESISTANCE IN 4 CASES OF MILD JUVENILE DIABETES

A Preliminary Report

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ABSTRACT Thorell J I Nilsson K O and Hager A (Departments of Nuclear Medicine and Paediatrics University Clinics General Hospital Malmo and the Department of Paediatrics University Hospital Linkoping Sweden) Absence of insulin resistance in 4 cases of mild juvenile diabetes A preliminary report Acta Paediatr Scand 64 248 1975 —Four children with a mild non insulin requiring diabetes were studied They had no insulin response at intravenous glucose tolerance test When insulin was infused at a rate which simulated a normal early insulin response to intravenous glucose blood glucose decreased to the same extent as it did in healthy subjects When a normal early insulin response was simulated during the intravenous glucose tolerance test the glucose assimilation rate was normalized These results suggests that a peripheral resistance to insulin is unlikely in mild juvenile diabetes and that the primary defect is a deficient release of insulin

KEY WORDS Juvenile diabetes early insulin response glucose assimilation rate

Mild forms of diabetes mellitus being asymptomatic and manifesting themselves by intermittent glucosuria by a slightly elevated fasting blood glucose and by a decreased glucose tolerance are most often encountered in early stages of the maturity onset type of the disease In recent years attention has been repeatedly drawn to the occurrence of such mild forms also in children and adolescents (6 7 10 11 14) In contrast to the classical juvenile diabetes which is characterized by an absolute insulin deficiency insulin production is retained with either an exaggerated insulin response to glucose or a relative insulin deficiency with a delayed insulin release These findings have been taken as evidence for insulin resistance as a factor in the pathogenesis of mild diabetes in children

Judging from our experience with intravenous glucose tolerance tests in non obese children with mild diabetes very low or no insulin responses are common We therefore tried to find out whether the responses were low because of a primary impairment of the release of insulin with a preserved sensitivity to the minimal amounts of insulin secreted or merely because of exhaustion of the β cells secondary to peripheral insulin resistance (3 12) The experimental model used was based on the intravenous glucose tolerance test and estimated the effect on blood glucose of a simulated early insulin response We have previously shown that the insulin sensitivity of healthy persons could be estimated by means of this experimental model (17) and that it varied inversely with the early insulin response of the individual

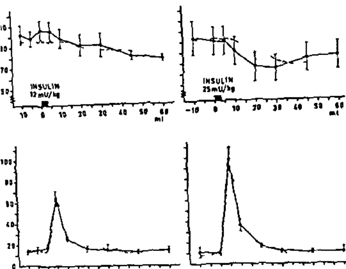


Fig 1 Effect of insulin infusion of two different doses on plasma insulin and blood glucose (mean±S E M) in cases of mild diabetes (—) Curves ( ) for 6 healthy persons are given for comparison To make the glucose levels comparable between the two groups a slow infusion of glucose (25 mg/min) was given to the persons of control group throughout the test as has been reported in detail (17)

In the present investigation four children with mild diabetes were studied

CASE REPORTS

Relevant data on carbohydrate metabolism and the time interval between the detection of glucosuria and the studies are given in Table 1 All four cases had normal height and weight development according to Swedish standards (?)

Case 1

A 10-year-old boy oldest of four healthy siblings No family history of diabetes Routine examination by the school physician revealed intermittent glucosuria The examination revealed no symptoms or physical abnor malities Weight 9 kg Height 136 cm He was given a diet of ?000 calories and treated with chlorpropamide 0 ?5 g a day on which treatment he remained well and aglucosuric for 6 months Thereafter in ulin therapy had to be instituted

Case 2

A 10-year-old girl youngest of three healthy siblings Her maternal aunt had diabetes No other known cases of diabetes in the family Routine examination of the urine because of an upper respiratory tract infection re vealed glucosuria No symptoms of diabetes Physical examination showed nothing remarkable Weight 35 kg Height 144 cm She was gi en a diet of 2?00 calories and treated with chlorpropamide 0 5 g a day She has remained well and aglucosuric during an observation period of 18 months

Case 3

A 14 year-old boy with no family history of diabetes Glucosuria diagnosed at examination because of a minor viral infection No symptoms of diabetes and no physi cal abnormalities Weight 63 kg Height 178 cm He was treated with glibenclamide ? 5 mg a day for one week Fasting blood glucose was normalised and glucosuria disappeared He has then remained well on a diet of 2600 calories during an observation period of 15 months

Case 4

A 9 year-old girl with no family history of diabetes Glucosuria was discovered at a routine control after un complicated measles No symptoms of diabetes and no physical abnormalities Weight 31 kg Height 144 cm She was given a diet of 2000 calories and remained well and aglucosuric for 19 months Thereafter insulin thera py had to be instituted

METHODS

All studies were performed before the institution of any therapy but for dietary regulation except in case 3 where the patient had been given glibenclamide for one week 2 months before the investigation

Simulated early insul n response test (SERT) was per formed as described in detail elsewhere (17) It meas ures the decrease in blood glucose after transient hyper insulinaemia at physiologic levels in the post hepatic circulation The hyperinsulinaemia is induced by the in fusion of insulin (Porcine insulin at neutral pH Actra pid® NOVO A/S Copenhagen) for 3 minutes and simu lates an insulin response to an intravenous glucose load

Table 1 *Glucose and insulin data on 4 children with mild juvenile diabetes and on a control group of 6 young healthy subjects*

Patient	Interval between the detection of glucosuria and the studies	Fasting blood glucose (mg/100 ml)	K_G	Basal plasma insulin (μU/ml)	Max insulin value during GTT (μU/ml)	Decrease in blood glucose during SERT (mg/100 ml) Dose of insulin 12 mU/kg	Dose of insulin 25 mU/kg
1	10 days	53–122	0 85	7	10	21	30
2	14 days	80–114	0 56	18	20	6	23
3	9 weeks	90–115	0 50	31	31	12	25
4	13 months	80–110	0 51	19	18	21	26
Controls[a] (Mean±S D)		79±3	1 4±0 2	9±2	62±24	19±4	27±3

[a] The results from studies of the control group are reported in detail elsewhere (17)

(18) Each patient was tested twice first with a dose of insulin of 12 mU/kg bodyweight and 60–80 minutes later with a dose of 25 mU/kg bodyweight Plasma insulin determined with a radioimmunoassay (8) and blood glucose determined with a glucose oxidase method (9) were measured every 5 minutes in samples collected from 15 minutes before until 60 minutes after the infusion of insulin The decrease in blood glucose was estimated as the difference between the mean of the pre injection values and the mean of the two lowest consecutive values after each injection

The intravenous glucose tolerance test (GTT) was performed with 0 5 g glucose per kg bodyweight injected as a 30% solution for 3 minutes The glucose assimilation rate was estimated from the graph of the log blood glucose versus time and expressed as per cent fall per minute (K_G)

In Case 3 a second GTT was combined with SERT by the simultaneous infusion of glucose and insulin (25 mU/kg) at rates and in amounts described above starting the insulin and glucose infusions at the same moment In Case 4 this procedure was supplemented with a continuous infusion of insulin 0 3 mU/kg bodyweight and minute from 0 to 70 min

RESULTS

The infusion of insulin simulating the early insulin response in the post hepatic circula

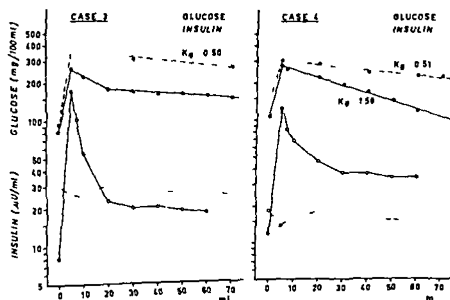


Fig 2 Blood glucose and plasma insulin during GTT ( ) and during GTT with a simulated insulin response (—) Left Case 3 in which the simulated response consisted of the early response only right Case 4 in which it contained both the early and the late phases

tion produced a peak in the plasma insulin with a maximum value of 64±15 μU/ml (mean±S D) following the smaller dose and 98±23 μU/ml after the larger dose (Fig 1) In all 4 cases the peaks were followed by significant decreases in blood glucose on average 15±7 mg/100 ml and 26±3 mg/100 ml respectively (Table I)

All four cases lacked insulin responses during the GTT and the K_G values were abnormally low When a GTT was performed simultaneously with the simulation of a normal early insulin response in Case 3 the slope of the glucose curve corresponded to a K_G of 1 7 during the first 20 minutes of the test after which the curve became notably flatter corresponding to a K_G of less than 0 5 (Fig 2) In Case 4 a GTT was performed simultaneously with a simulated early insulin response supplemented with a low rate continuous insulin infusion for 60 minutes This simulated a complete insulin response including the early as well as the late phases of the response During this test the K_G was 1 5

DISCUSSION

The early insulin response to intravenous glucose injection has been shown to be an important determinant of the glucose assimilation rate (13) It is reduced in most types of diabetes also in very mild forms with a late but high insulin response (15 16) Since the early response may be low in pre-diabetes (1 4 5) it is probably a function of the β-cell which is particularly susceptible to a diabetic influence The early insulin response to glucose was markedly reduced in the 4 children with mild diabetes studied

The present study showed that when a normal early insulin response was produced in mild juvenile diabetics by the infusion of insulin in such a way as to simulate a normal endogenous delivery rate of insulin to post hepatic circulation (17) the effect on the blood glucose was similar to that recorded by this procedure in young healthy persons These results indicate that resistance to insulin at least with regard to its peripheral effect on blood glucose does not seem to play any role in the pathogenesis of mild diabetes in children The restoration of a normal glucose assimilation rate when insulin was infused during the glucose tolerance tests simulating a normal insulin response suggests that the primary defect is a deficient secretion of insulin It remains to be shown whether the present findings apply to mild diabetes in general or only for a special form of mild diabetes occurring in children Long term follow up of such cases has shown that some of them progress to an insulin therapy dependent diabetes of juvenile type (7) as occurred in two of our cases

ACKNOWLEDGEMENT

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REFERENCES

1 Boden G Soeldner J S Gleason R E & Marble A J Elevated serum growth hormone and decreased serum insulin in prediabetic males after intravenous tolbutamide and glucose *J Clin Invest* 47 729 1968

2 Broman B Dahlberg G & Lichtenstein A Height and weight during growth *Acta Paed atr Scand* 30 1 1942

3 Camerini Davalos R A Biochemical and histological aspects of prediabetes *In* B S Leibel & G A Wrenshall (eds) *On the nature and treatme it of diabetes* New York 1965 Excerpta Medica Foundation p 657

4 Cerasi E & Luft R Insulin response to glucose infusion in diabetic and non-diabetic monozygotic twin pairs Gentic control of insulin response? *Acta Endocr* 55 330 1967

5 Colwell J A & Lein A Diminished insulin response to hyperglycemia in prediabetes and diabetes *Diabetes* 16 560 1967

6 Drash A Chemical diabetes mellitus in the child *Metabolism* 22 255 1973

7 Fajans S S Floyd J C Pek S & Conn J W Studies on the natural history of asymptomatic diabetes in young people *Metab lism* 22 327 1973

8 Heding L G A simplified insulin radioimmuno assay method *In* L Donato G Millhaud & J Sirchis (eds) *Labelled p oteins in tracer studies* Euratom Brussels 1966 p 345

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RESULTS

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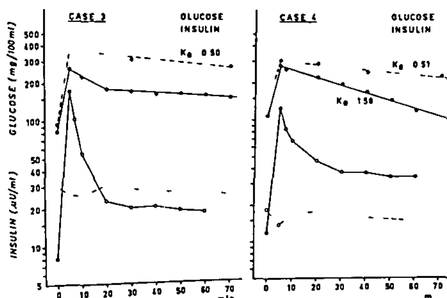


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SUBMAXIMAL BLOOD FLOW AND BLOOD VISCOSITY IN NEWBORN INFANTS

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ABSTRACT Bergqvist G and Zetterstrom R (Department of Paediatrics Karolinska Institutet St Goran s Children s Hospital Stockholm Sweden) Submaximal blood flow and blood viscosity in newborn infants Acta Paediatr Scand 63 253 1974 —In new born infants with varying hematocrit values the submaximal blood flow has been studied by the use of strain gauge plethysmography under standardized conditions Submaximal flow was defined as the flow obtained after 4 minutes of suprasystolic occlusion With in creasin hematocrit there was a decreasing maximal flow Capillary filtration coefficient seemed to decrease with increasing hematocrit The relation between circulatory failure in newborns due to abnormaly high hematocrit and low capacity to increase blood flow upon demand has been discussed

KEY WORDS Submaximal flow blood viscosity newborn infants hematocrit secondary polycythemia pl tysmography

In newborn infants there is a good correla tion between blood viscosity and hematocrit even if the viscosity increases much faster than the hematocrit (3) However the resting muscular blood flow as measured in the calf and foot has been found to be unaltered by variations of blood viscosity and hema tocrit (4) Even if the hematocrit is extremely high the resting flow remains normal How ever it is a welll nown fact that some new born infants with high hematocrit (secondary polycythemia) develop symptoms of circula tory failure (12 15) If elevated blood vis cosity is contributory the hypothesis may be advanced that maximal flow is reduced although resting flow does not vary with the hematocrit To elucidate this possibility the following study was made

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Thi tudy h s be n suppo ted by grant from Crown Prince L wis Fou idat c for Child Health and Axel T elm n Found t on

MATERIAL AND METHODS

All infants had normal deliveries with Apgar scores of 9 or 10 at 1 minute Nothing abnormal was found during the first week of life

A The babies of groups A1 and A2 were included in a pilot study

A1 Ten 1-day-old full term infants with a *mean birth* weight of 3550 g (S D 495 g) and a mean gestational age of 40 4 weeks (S D 1 8 weeks)

A2 Eleven 3-day old infants with a mean birth weight of 3 370 g (S D 710 g) and a mean gestational age of 39 5 weeks (S D 2 3 weeks) The range for gestational age for these pilot groups was 37 to 42 weeks

B *Fourteen 1-day-old* (20 to 36 hours) term infants with a mean birth weight of 3490 g (S D 410 g) and a mean gestational age of 39 9 weeks (S D 1 0 weeks) All infants had normal deliveries with Apgar scores of 9 or 10 at 1 m nute and were regarded as healthy

When the infant in luded in groups A1 and A2 were stud ed they were lying in ordinary cots with double blankets Room temperature was 23 0 C and the tem perature under the blankets at the skin 32 0°C The in fants belong ng to group B were dressed and placed in an incubator at a temperature of 32 0 C Most probably all of the babies examined were kept within the neutral temperature zone where muscular blood flow (i e calf and foot) is reported to be stable (7) The infants in group B were examined 1½ hours after feeding

9 Hjelm M & de Verdier C H A methodological study of the enzymatic determination of glucose in blood *Scand J Clin Lab Invest 15* 415 1963
10 Jackson R L Insulin treatment of children with late stage of chemical diabetes *In* R A Camerini Davalos & H S Cole (eds) *Early Diabetes* Academic Press New York and London 1970 p 401
11 Johansen K Mild diabetes in young subjects *Acta Med Scand 193* 23 1973
12 Kipnis D M Insulin secretion in diabetes mellitus *Ann Int Med 69* 891 1968
13 Lerner R I & Porte D Jr Relationships between intravenous glucose loads insulin responses and glucose disappearance rate *J Clin Endocr 33* 409 1971
14 Rosenblom A I Insulin responses of children with chemical diabetes mellitus *N Engl J Med* 282 1228 1970
15 Seltzer H S Allen E W Herron A L Jr & Brennan M T Insulin secretion in response to glycemic stimulus Relation of delayed initial release to carbohydrate intolerance in mild diabetes mellitus *J Clin Invest 46* 323 1967
16 Simpson R G Benedetti A Grodsky G A Karam J H & Forsham P H Early phase of insulin release *Diabetes 17* 684 1968
17 Thorell J I Effect of transient elevation of plasma insulin within physiologic levels (Simulated early insulin response) on blood glucose *J Clin Endocr 37* 423 1973
18 Thorell J I Nosslin B & Sterky G Estimation of the early insulin response to intravenous glucose injection *J Lab Clin Med* 82 101 1973

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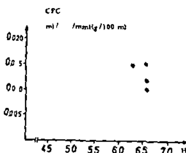


Fig 3 Relation between capillary filtration coefficient and hematocrit in eleven 1-day-old infants

with a hematocrit which is normal for age (4) However maximal flow has been demonstrated to diminish with increasing hematocrit and is thus in agreement with the findings of Bollinger & Luthy in adults (5) The absolute values of flow are lower than those reported by Celander et al (6 8) after a 10-minute occlusion period and at a temperature of 40 C (mean 47 ml/min/100 ml tissue) For our purpose however we have considered a submaximal flow induced by an occlusion period of only 4 minutes and a lower temperature to be sufficient

Celander et al have found the range for CFC to be 0 005 to 0 020 ml/100 ml tissue/min/mmHg with a mean of 0 011 ml (9) Our results are in fair agreement with these findings Celander et al (9) were unable to demonstrate any correlation between flow and CFC However at maximal flow in working muscle of cats CFC is tripled (14) In our studies CFC seemed to decrease with increasing hematocrit One reason for this may be altered pre and postcapillary resistances

Thus the reserve capacity for flow in muscles diminishes with increasing hematocrit In newborn polycythemic infants isovolemic hemodilution a procedure which has a considerable effect on blood viscosity/hematocrit is followed by a marked improvement of renal function (1) Circulatory failure in polycythemic newborn infants mainly affects central nervous system the lungs and kidneys (12 15) It may be speculated upon whether this may happen when the reserve flow capacity is very low and there is no further ability to compensate for an increasing blood viscosity From the results of this study of muscle flow it may be assumed that such an inability may occur when the hematocrit exceeds a value of 70% and may be more definite when it reaches 75 to 80% It may be pointed out however that this calculation is very hypothetical since the shape of the extrapolated curve cannot be predicted The critical value may also be different in various vascular areas due to variations of vascular geometry and function Other factors such as acidosis low blood pressure cardiopulmonary shunts and erythrocyte aggregation may also play a significant role for the development of circulatory failure in polycythemic newborn infants (10 11) That cardiac shunts may play an important role is demonstrated in experiments on newborn lambs (11) where the increased pulmonary resistance caused shunting through ductus arteriosus and secondary through foramen ovale

REFERENCES

1 Aperia A Bergqvist G Broberger O Thodenius K & Zetterstrom R Renal function in newborn infants with high hematocrit before and after isovolemic hemodilution *Acta Paed atr Scand* 63 878 1974
2 Berg K & Celander O Circulatory adaptation in the thermoregulation of fullterm and premature newborn infants *Acta Paed atr Scand* 60 78 1971
3 Bergqvist G Viscosity of the blood in the newborn infant *Acta Paediatr Scand* 63 858 1974
4 Bergqvist G & Zetterstrom R Blood viscosity and peripheral circulation in newborn infants *Acta Paediatr Scand* 63 865 1974
5 Bollinger A & Luthy E Blood viscosity and blood flow in the human forearm *Helv Med Acta* 34 fasc 3 55 1968
6 Celander O Blood flow in the foot and calf of the newborn *Acta Paediatr Scand* 49 488 1960
7 Celander O & Thunell C The mercury in rubber strain gauge for instruments of blood pressure and peripheral circulation in the newborn infants *Acta Paed atr Scand* 50 505 1961
8 Celander O & Mårild K Reactive hyperemia in the foot and calf of the newborn infant *Acta Paediatr Scand* 51 554 196
9 — Regional circulation and capillary filtration in relation to capillary exchange in the foot and calf

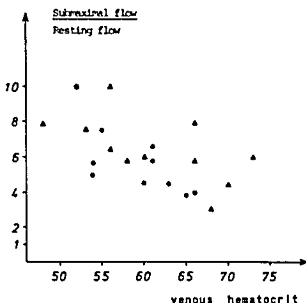


Fig 1 Relationships between hematocrit and the submaximal/resting blood flow ratio in 21 newborn infants Ten were 1 day old (●) and eleven were 3 days old (▲)

Maximal flow (i e submaximal flow) was measured in the calf and defined as the flow after 4 min of suprasystolic occlusion with a pressure of 150 to 200 mmHg and 5 to 10 sec after cessation of the occlusion The usual occlusive pressure for flow measurements was 40 mmHg The strain gauge was placed on the calf at the largest circumference

Capillary filtration coefficient (CFC) was measured in 9 of the babies included in group B prior to the determination of arterial blood pressure and maximal flow The occlusive pressures were 20 or 30 mmHg and 40 mmHg respectively Precapillary resistance was assumed to be 80% (9 14)

CFC was also determined in the same way in another two healthy 1-day old infants with body weight 3 490–3 770 g and venous hct 66–72%

A strain gauge plethysmograph was used (7 17) (LPM periquant II) The technical aspects of this equipment have been described by Gutman (12) and Schreiber (16) Hematocrit was determined as in earlier studies (3)

RESULTS

The relationships between hematocrit and the maximal/resting flow ratio in groups A1 and A2 are shown in Fig 1 Since statistical analysis with the *t* test shows no difference between the two groups for hematocrit maximal flow/resting flow ratio birth weight or gestational age they were treated as one single group However there was a more pronounced deviation for the flow ratio on day 3 Therefore all infants in group B were examined on the first or second day i e 20 to 36 hours after birth

Correlation analysis of the hematocrit/flow ratio shows this to be significant ($p<0.01$) both by ordinary analysis ($r=-0.59$) and by Spearman s rank correlation

The results for group B are shown in Fig 2 The maximal flow/resting flow ratios varied in the same manner Correlation analysis shows a significant correlation ($p<0.01$) ($r=-0.74$) both by ordinary analysis and by Spearman s rank correlation test for both the ratios and the absolute values Additional studies in a few infants with perinatal asphyxia showed lower ratios as well as lower maximal flows than was expected from the hematocrit Resting flow did not show any correlation to the hematocrit The mean resting flow was 5 3 (S D 1 1) ml/min/100 ml tissue

Fig 3 shows the relation between CFC and hematocrit Spearman s rank correlation on the 11 infants was almost significant ($p<0.05$) Mean and S D for CFC was 0 013 and 0 004 ml/min/100 ml tissue/mmHg respectively

DISCUSSION

This study confirms the results of earlier studies in which the normal resting blood flow was found to be the same in newborn infants with a high hematocrit as in those

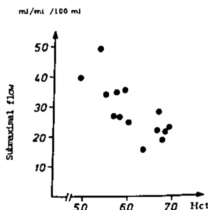


Fig 2 Relation between submaximal/blood flow and hematocrit in fourteen 1-day-old infants

Acta Pædiatr Scand 64 257–263 1975

TEMPERATURE REGULATION IN CHILDRFN DURING EXERCISE

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ABSTRACT Gullestad R (Institute of Work Physiology and Institute of Zoophysiology University of Oslo Oslo Norway) Temperature regulation in children during exercise Acta Paediatr Scand 64 257 1975.—Rectal and skin temperature and sweat rate were measured in eight 11 year-old boys exercising one hour on a bicycle ergometer at each of three different work loads. Rectal temperature rose according to the relative work load and reached a steady state level after a shorter time than had been previously observed in adult subjects. A good relationship was observed between the levelling off value of rectal temperature during work and the relative work load but the value of rectal temperature at rest just before the start of work affects this steady state value The average skin temperature was kept fairly constant during exercise except on the heaviest work load (70%) during which it rose about 1 C mainly as a result of the rise in skin temperature on the arm hand and thigh Temperature increases on skin locations in general were minimal except on the back where the temperature decreased slightly Sweat rate showed a close relationship to the absolute work load and in this report the conclusion is supported that the 11 year-old boys regulate their body temperature during exercise at constant work load in the same way as do adult subjects

KEY WORDS temperature regulation rectal temperature skin temperature sweat rate exercise children

During prolonged muscular exercise the deep body temperature increases to a constant level which is directly proportional to the work load performed (15 16 20) Using different types of work i e arm work positive and negative leg work B Nielsen showed that the increase in the deep body temperature was related to the oxygen uptake rather than to the work load performed (11 12 13) Åstrand (21) and more recently Saltin & Hermansen (17) obse ved that the constant level of the core temperature was set according to the relative work load i e oxygen uptake expressed in per cent of the individuals maximal oxygen uptake and not to the absolute work load However our knowledge of temperature regulation during exercise in man is almost entirely based on experiments with adult subjects The aim of the present study was to measure the skin and deep body temperature during prolonged severe exercise in 11 year old boys

MATERIALS AND METHODS

Eight boys 10–11 years of age were selected from a group of ?? volunteers representing a school class participating in a broader longitudinal study of physical growth and development In all classes the consent of the subject was obtained and the parents were well informed about the nature of the investigation The subjects were given health examinations by a physician prior to their participation in the project Pertinent data describing the subjects are given in Table 1 All work experiments were performed in a climate chamber at ±1 C ambient temperature The air movement was 0 5 m/sec and the relative humidity varied between 35 and 55% The exercise was performed on an electrically

of the newborn infant *Acta Paediatr Scand 51* 385 1962
10 Dintenfass L *Blood microrheology* Butterworths London 1971
11 Fouron J C & Hébert F The circulatory effects of hematocrit variations in normovolemic newborn lambs *J Pediatr* 82 995 1973
12 Gross G P Hathaway W E & McGaughey H R Hyperviscosity in the neonate *J Pediatr 82* 1004 1973
13 Gutman J Moderne Gerätetechnik fur Venenverschlussplethysmografie *Herz Kreisl 3* 365 1971
14 Kjellmer I The effect of exercise of the vascular bed of skeletal muscle *Acta Physiol Scand 62* 18 1964
15 Kontras S B Polycythemia and hyperviscosity syndromes in infants and children *Pediatr Clin North Am 19* 919 1972
16 Schreiber H Die Venenverschluss pletysmographie in der Praxis *Medicinal marktlacta medicotechnica 18* 37 1970
17 Whitney R J The measurement of volume changes in human limbs *J Physiol* (London) *121* 1 1953

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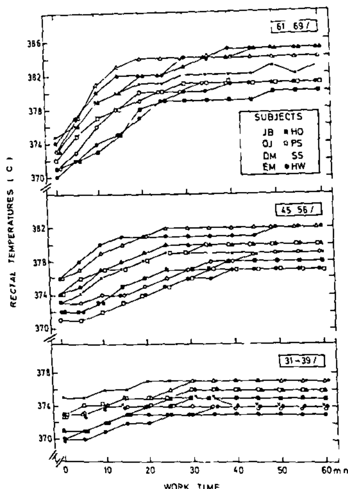


Fig 1 Rectal temperature during exercise at different relative work loads

related to the magnitude of the work load performed

It should be noted that the resting value of T varied considerably from one subject to another i e from 36 9 to 37 6 C This variation markedly affected the steady state level of T This was especially clear from the experiments in which P S and S S worked twice P S on a work load of 50% and S S on 30% (Fig 1) The steady state value of T was 37 7 and 38 0°C respectively at 50% but ΔT was 0 6 C in both experiments At 30% the values were 37 4 and 37 7°C while ΔT_{re} was 0 1 and 0 2°C Fig 2 clearly demonstrates for the whole material the relationship between T_{re} at rest

In Fig 3 the rectal temperature at the end of each experiment is plotted as a function of the relative work load i e oxygen uptake in per cent of the individuals maximal oxygen uptake T_{re} rose with increasing work load although considerable variations from

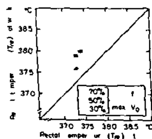


Fig 2 The relationship between rectal temperature at rest and the levelling off value during work

Table 1 *Physical characteristics and physiological responses to maximal exercise on bicycle ergometer*

Subject	Age	Height (cm)	Weight (kg)	Resting oxygen uptake (l/min)	Maximal oxygen uptake (l/min)	Maximal oxygen uptake (ml/min kg)	Highest measured heart rate (beats/min)	Highest measured lactic acid in blood (mg/100 ml)
J B	11	154	37 5	0 19	2 23	59 5	198	80
O J	10	153	40 5	0 25	1 91	47 2	–	61
D M	11	148	38 6	0 22	1 89	49 0	200	98
E M	10	149	35 2	0 23	1 76	49 1	198	56
H O	10	150	35 2	0 21	2 23	63 4	204	66
P S	11	138	30 6	0 17	1 60	52 3	202	113
S S	10	152	38 2	0 20	1 98	51 8	182	–
H W	11	141	35 0	0 20	1 89	54 0	196	67

braked bicycle ergometer and the pedal rate was kept at 60 rpm using a metronome Oxygen uptake was determined with the Douglas bag method The gas volume was measured in a spirometer and the gas analyses performed according to the method of Scholander (18) The heart rate was determined by a conventional ECG recorder and the blood lactate concentration was measured in samples taken from a prewarmed clean and dry fingertip and analysed according to the Strom modification (19) of the colorimetric method of Barker & Summerson (1)

The rectal temperature (T) was measured at three depths i e 4 8 and 12 cm from the external sphincter using copper constantan thermocouples mounted in a 7 mm plastic tube The average of the three measured temperatures was applied as T_r

The skin temperature was determined at 7 different locations (forehead upper arm hand chest back leg and thigh) with a skin thermocouple The average skin temperature ($\bar{T}$) was calculated according to the method described by Hardy & Du Bois (6)

The temperatures were read continuously on a 24 channel Speedomax recorder with adjustable zero and adjustable range (AZAR unit) The measurements were considered to be accurate to within ±0 05 C A calibration curve was determined for each thermocouple Furthermore the thermocouples for T_{re} were calibrated immediately after each experiment The calibration of the skin thermocouples was checked before in the middle and fter the experimental period Always the same curves of calibration were obtained

The total weight loss of the subjects was measured by a balance sensitive to ±10 g The sweat secretion was calculated from the total weight loss by subtracting the weight loss due to the respiratory gas exchange and the respiratory water loss The heat production (H) during the experiment was calculated according to $H=M-W$ where M=the metabolic energy liberation during exercise calculated from the oxygen uptake using a caloric equivalent of 4 9 kcal per litre of oxygen and W=the external work converted to heat using the mechanic equivalent of 427 kpm per kcal

PROCEDURE

The subjects arrived at the laboratory on 5 different days The first 2 days were used to estimate the relationship between oxygen uptake and work load during submaximal exercise and to determine the maximal oxygen uptake The maximal oxygen uptake was measured on the bicycle ergometer and in general the method described by Hermansen & Saltin (7) was used

On the third fourth and fifth experimental day the subjects performed 1 hour on the bicycle ergometer at a work load corresponding to approximately 30 50 and 70% of the individuals maximal oxygen uptake respectively

The subjects came to the laboratory about nine o clock after having consumed a light meal 1-2 hours before Prior to the exercise the subjects rested for at least 30 min At the end of this period the resting oxygen uptake was determined

The rectal and skin temperatures were measured during rest and at every fifth minute during exercise Oxygen uptake was measured twice during the 60 min work period i e between the 2 h and the 27th min and between the 50th and 52th min Heart rate was recorded each minute and the body weight was measured before and immediately after termination of the exercise

RESULTS

Fig 1 shows the individual values for the rectal temperature (T_{re}) at different time intervals during a 60 min bicycle exercise at three different work loads representing about 30 (31–39) 50 (45–56) and 70 (61–69)% of the individuals maximal oxygen uptake It can be seen that T_{re} increased in all subjects during the first 20–40 min of the exercise period before reaching a constant level The duration of the rise in the T_{re} was no

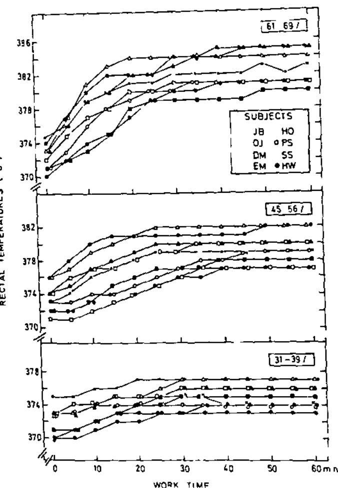


Fig 1 Rectal temperature during exercise at different relative work loads

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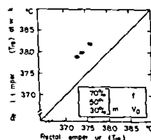


Fig 2 The relationship between rectal temperature at rest and the levelling off value during work

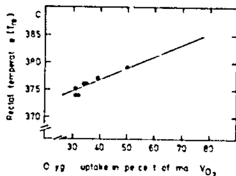


Fig 3 The levelling off value of rectal temperature during exercise as a function of the relative work load The regression line $y = 0\ 21x + 36\ 9$ is drawn

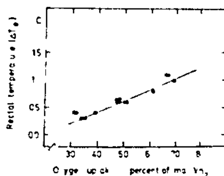


Fig 4 The rise in rectal temperature from resting value during exercise as a function of relative work load The regression line $y = 0\ 21x - 0\ 42$ is drawn

SKIN TEMPERATU ES (°C)
FOREHEAD
CHEST
ARM
HAND
70%
50%
30%
of ma V_{O_2}
WORK TIME
a

SKIN TEMPERATURES (°C)
BACK
THIGH
LEG
WORK TIME
b

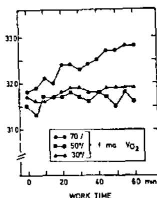


Fig 6 Average skin temperature during exercise at different relative work loads Each curve represents the mean of values from eight subjects

one subject to another were observed Figs 2 and 4 show that these variations were markedly reduced when the increase in body temperature was expressed as ΔT_{re} i e the increase in T from the resting value Regression lines were calculated and drawn in Figs 3 and 4 but no significant difference in residual variance around the regression line was found in the two situations

Fig 5 gives the skin temperatures at various skin locations during exercise at the three different work loads Each curve represents the average skin temperature of the eight subjects It can be seen from the illustration that the skin temperature on the forehead and chest remained fairly constant during the exercise period and also at the various work loads On the other hand the skin temperature of the arm hand thigh and leg increased and the skin temperature of the back decreased during the exercise period Variation in the work load caused the skin temperature to differ considerably at the various skin locations

The average skin temperature (T) is presented in Fig 6 It shows that only the highest relative work load produced an increase in the average skin temperature

Fig 5 Skin temperatures during exercise at different relative work loads Each curve represents the mean of values from eight subjects

Fig 7 illustrates the relation between sweat rate and the absolute work load The sweat rate increased almost linearly with increased work load

DISCUSSION

The results demonstrate that in 11 year-old boys the rectal temperature (T_{re}) rises during exercise according to the relative work load and reaches a steady state level after 20–40 min of work (Fig 1) Previous studies in adults have shown that this levelling off is accomplished after 40–50 min of work (9 10 14 15) or after approximately 58 min of work (3) during exercise at constant work loads This difference can be explained by the greater body mass of the latter subjects in relation to the boys The equilibrium between the temperature in the different parts of the body may be reached more slowly when the mass is greater

The steady state value of T_{re} is depending on the relative work load (expressed in per cent of the subjects maximal oxygen uptake) (Figs 1 and 3) But the T_r at the beginning of the exercise markedly affects the steady state value of the T_{re} This is clearly demonstrated by the experiments in which P S

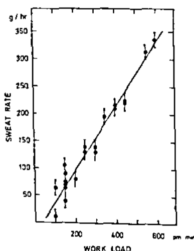


Fig 7 Sweat rate during one hour of exercise at different absolute work loads The line of regression $y = 0\ 59x - 23\ 7$ is drawn

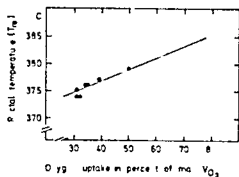


Fig 3 The levelling off value of rectal temperature during exercise as a function of the relative work load The regression line y = 0 21x + 36 9 is drawn

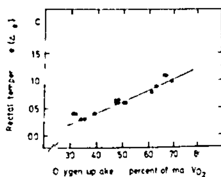


Fig 4 The rise in rectal temperature from resting value during exercise as a function of relative work load The regression line y = 0 [illegible]1x − 0 4[illegible] is drawn

SKIN TEMPERATURES (°C)
FOREHEAD
CHEST
ARM
HAND
70% / 50% / 30% of max VO_2
WORK TIME
a

SKIN TEMPERATURES (°C)
BACK
THIGH
LEG
70% / 50% / 30% of max VO_2
WORK TIME
b

al circulation control Sweat rate shows s expected a clear relationship to the absolute work load performed (Fig 7)

Although minor differences are observed the data appear to support a conclusion that 1 year-old boys regulate their body temperature during exercise at constant work load n the same way as do adult subjects

ACKNOWLEDGEMENTS

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REFERENCES

1 Barker S B & Summerson W M The colorimetric determination of lactic acid in biological material *J Biol Chem 138* 535 1941
2 Bevegård B S & Shepherd J T Reaction in man of resistance and capacity vessels in forearm and hand to leg exercise *J Appl Physiol 21* 123 1966
3 Clasing D & Laumann U Das Verhalten der Körpertemperatur bei Fahrradergometerarbeit im Bereich der Ausdauergrenze (P W C 30) *Int Z Angew Physiol* 26 475 1970
4 Ekblom B Greenleaf C J Greenleaf J E & Hermansen L Temperature regulation during exercise dehydration in man *Acta Physiol Scand 79* 475 1970
5 Grimby G Exercise in man during pyrogen induced fever *Scand J Clin Lab Invest 14* Suppl *67* 1962
6 Hardy J D & Du Bois E F The technic of measuring radiation and convection *J Nutr 15* 461 1938
7 Hermansen L & Saltin B Oxygen uptake during maximal treadmill and bicycle exercise *J Appl Physiol* 26 31 969
8 Kamon E & Belding H S Dermal blood flow in the resting arm during prolonged leg exercise *J Appl Physiol* 26 317 1969
9 Lind A R A physiological criterion for setting thermal environmental limits for every-day work *J Appl Physiol 18* 51 1963
10 — Physiological effects of continuous or intermittent work in the heat *J Appl Physiol 18* 57 1963
11 Nielsen B Regulation of body temperature and heat dissipation at different levels of energy—and heat production in man *Acta Physiol Scand 68* 215 1966
12 — Thermoregulatory responses to arm work leg work and intermittent leg work *Acta Physiol Scand 72* 25 1968
13 — Thermoregulation in rest and exercise *Acta Physiol Scand 76* Suppl *323* 1969
14 Nielsen B & Nielsen M Body temperature during work at different environmental temperatures *Acta Physiol Scand 56* 1 0 1962
15 Nielsen M Die Regulation der Körpertemperatur bei Muskelarbeit *Skand Arch Physiol 79* 193 1938
16 Robinson S Physiological adjustments to heat in L. M Newburg (ed) *Physiology of Heat Regulation and the Science of Clothing* Saunders Philadelphia 1959
17 Saltin B & Hermansen L Esophageal rectal and muscle temperature during exercise *J Appl Physiol 21* 1757 1966
18 Scholander P F Analyzer for accurate estimation of respiratory gases in one half cubic centimeter samples *J Biol Chem 167* 235 1947
19 Strøm G The influence of anoxia on lactate utilization in man after prolonged muscular work *Acta Physiol Scand 17* 440 1949
20 Wyndham C H Bonwer W v d M Devine M G & Paterson H E Physiological responses of african laborers at various saturated air temperatures wind velocities and rates of energy expenditure *J Appl Physiol* 5 290 1952
21 Åstrand I Aerobic work capacity in men and women with special reference to age *Acta Physiol Scand 46* Suppl *169* 1960

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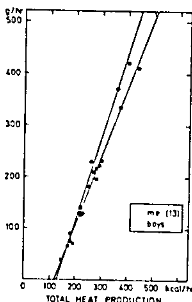


Fig 8 Sweat rate during one hour of exercise as a function of total heat production The results obtained by B Nielsen (13) for adult subjects and the results for the boys in the previous study are plotted Regression lines $y=1.64x-212.67$ for the former and $y=1.33x-158.96$ for the latter subjects are drawn

and S S performed the same work load twice (Fig 1) The ΔT_{re} was identical at the same work load but the levelling off value of T_{re} was higher when the resting value was higher Fig 2 demonstrates the same trend in all the work experiments These observations are in agreement with the work of Nielsen (15)

Although Figs 3 and 4 give the impression that a better prediction can be given for ΔT_{re} than for the T_{re} during work from relative work loads statistical evidence does not support this point of view In experiments made with subjects exercising in the chill period of pyrogen induced fever (5) however exercise is accompanied with a rise in T_{re} and this ΔT_{re} is equal to the T_{re} during no fever if the work load is equal

At the same relative work load the level of T_{re} during continuous exercise seems to be lower in 11 year old boys than in 20–30 year old subjects In the work of Saltin & Hermansen (17) the subjects attained a T_{re} of 38 1°C at a relative work load of 50% compared with 37 9°C in the boys of the present study even though the mean value of T_{re} in the former subjects prior to exercise was 0 6°C lower than that of the boys The difference between the two mean rectale temperatures at work was found to be significant at the 0 02 level according to a t test applied to the data representing the two groups of subjects

In contrast to the skin temperatures (T_s) of head and trunk the T_s of the extremities rises during exercise (Fig 5) According to Bevegård & Shepherd (2) and Kamon & Belding (8) blood flow through the skin of the arms increases during leg work In the present study the T_s of upper arms rose 3 2°C in average at a work load of about 70% This rise probably indicates an enhanced blood flow which causes an increase in the heat dissipation from the arms

Except from exercise at the highest load during which the mean value of T_s rose 1°C the average skin temperature is kept fairly constant (Fig 6) In other works (4 11 17) the T_s is shown to decrease about 0 5°C during exercise in 20–30 year old subjects working on a work load of 60–70%

Assuming that the central blood temperature is kept constant when T_{re} has reached a steady state level this continuous rise in T_s even after 20–40 minutes exercise on 70% work load when environmental temperature air velocity and relative humidity are kept nearly constant can be explained either by enhanced skin blood flow or by reduced sweat rate

In Fig 8 the sweat rate from the experiments with the boys are presented as a function of heat production together with results obtained for adult subjects working on a constant work load (13) Regression lines were calculated for the two groups but the regression coefficients of the two lines were not significantly different when compared by applying the t test to the data Since the 11 year old boys do not deviate from the adult subjects with regard to sweat rate the continuous rise in T_s on the 70% work load may indicate somewhat different periph

eral circulation control Sweat rate shows as expected a clear relationship to the absolute work load performed (Fig 7)

Although minor differences are observed the data appear to support a conclusion that 11 year-old boys regulate their body temperature during exercise at constant work load in the same way as do adult subjects

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1 Barker S B & Summerson W M The colorimetric determination of lactic acid in biological material *J Biol Chem 138* 535 1941

2 Bevegård B S & Shepherd J T Reaction in man of resistance and capacity vessels in forearm and hand to leg exercise *J Appl Physiol 21* 123 1966

3 Clasing D & Laumann U Das Verhalten der Korpertemperatur bei Fahrradergometerarbeit im Bereich der Ausdauergrenze (P W C $_{30}$) *Int Z Angew Physiol 26* 475 1970

4 Ekblom B Greenleaf C J Greenleaf J E & Hermansen L Temperature regulation during exercise dehydration in man *Acta Physiol Scand 79* 475 1970

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7 Hermansen L & Saltin B Oxygen uptake during maximal treadmill and bicycle exercise *J Appl Physiol 26* 31 969

8 Kamon E & Belding H S Dermal blood flow in the resting arm during prolonged leg exercise *J Appl Physiol 26* 317 1969

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GROWING PAINS AND RESTLESS LEGS

KARL AXEL EKBOM

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ABSTRACT Ekbom K A (Department of Neurology University Hospital Uppsala Sweden) Growing pains and restless legs Acta Paediatr Scand 64 264 1975.—Growing pains is a common and distressing symptom in children but has aroused little interest There is a similarity between growing pains and the painful form of restless legs but it is not known if these two conditions are identical I have recently observed a family in which the mother has growing pains since her childhood The pain persists in adult age which is unusual She also has typical restless legs Her three sons have severe growing pains The study of this family has convinced me that growing pains and restless legs are different conditions

KEY WORDS Growing pains restless legs Ekbom s syndrome

Growing pains in children are common and cause much suffering Little has been written on this subject however For a review of the literature I refer to Øster (5) Growing pains and restless legs (Ekbom s syndrome) resemble each other Wersall (4) and Brenning (1) considered that the two conditions are identical or related I discussed this question in my monograph on restless legs (2) but did not come to any definite conclusion Twenty five years later I was still in doubt I then wrote that growing pains resembled the painful form of restless legs but added that the intensity of pain is probably greater in growing pains (3)

Most patients with restless legs have only disagreeable creeping sensations in their legs but some have real pain No pathological signs are found in either disease

I have seen several hundred patients with restless legs but until recently no adult with growing pains I have now, however observed a woman who has suffered from growing pains since her childhood She also has restless legs and so has been able to compare the two conditions

CASE REPORTS

Case 1

A married schoolteacher 37 years old has for several years had periods of creeping sensations in her legs between the knee and ankle There is no pain The discomfort usually appears an hour after she has gone to bed and wakes her up from sleep She must then move her legs but does not leave the bed After half an hour or at most one hour she falls asleep again The creeping sensations have never appeared during the day or the evening but only when she is in bed They are provoked by exertion during the day Two years ago she went on a cycling tour during her holiday Then she had creeping sensations at night but they ceased when the tour was over

She has three sons and is now pregnant again She has not had restless legs during her pregnancies

As long as she can remember she has suffered from growing pains It is a constant aching pain like toothache of unbelievable intensity The pain is felt deep inside the calves and usually only on one side at the time A few times it has appeared in both legs simultaneously and this was utterly disagreeable The pain occurs after two or three hours of sleep and wakes her up It is accompanied by a general distress She tosses about in bed but she is not compelled to move her legs She takes an aspirin (acetylsalicylic acid)

and then the pain disappears in 15 minutes She has always taken a tablet and for this reason does not know how long the ache would last if untreated When the pain ceases at first its intensity diminishes Then it disappears for a while (not more than one minute) and then recurs though less severely Then it disappears again and recurs still weaker In this way it continues until the ache is completely gone On rare occasions the pain occurs in the day but then it is rather mild

She has now the pain about once a month often the day before her menstruation It is often provoked by exertion for instance a stressing day in school or cycling When she was a child the pain appeared more frequently at least a few times every month

During her first three pregnancies she did not have growing pains more often than usual During her fourth pregnancy (now in her eight month) she has had growing pains only once In her opinion the creeping sensations (restless legs) are quite different from the ache (growing pains) She never has these symptoms at the same time The growing pains are also different from cramp

Both her parents had growing pains The paternal grandmother did not suffer from growing pains but her five sisters had much ache in their legs during child hood When the patient was a child they used to say *It is only* growing pains When we were children we also had such pains and there is nothing to do but to take an aspirin The patient s only brother had severe *growing* pains and so had her husband and his two brothers Only *the patient herself* has growing pains in adult age In all her relatives the pain disappeared after *childhood* The patie has three sons 12 10 and 7 *years old* and they ha all had intense growing pains since the age of one *year* *The pain* now seems to be milder than earlier

Case 2

The patient s eldest son 12 years old says that it is a severe ache deep inside the calf *usually* only on one side but sometimes in both legs at *the same time* It appears only after a few hours of sleep He wakes up and tosses in bed and moves his legs but that does not help *He does* not walk about on the floor *At last* he takes an aspirin and then the pain disappears after 15–30 *minutes* *The* pain occurs 2–3 times a month especially when he has been playing football or running

His two younger brothers describe their pains in the same way Their mother says *that* her youngest son cries of *pain* The frequency is the same in summer and winter The father who is a physician thought that the children consumed too many tablets He tried place bo but that d d not help Paracetamol gave quick *relief*

DISCUSSION

In restless legs there are disagreeable creep ing sensations deep inside the legs mostly between the knee and ankle occasionally also in the feet and thighs in rare cases also in the arms They cause an irresistible need to move the legs The symptoms appear during the afternoon when the legs are kept still as at the cinema in front of the television and in bed before or during sleep In mild cases they last 10–30 minutes In severe cases they last with *interruptions* almost the whole night Even then they usually disappear after 4 or 5 in the morning They hinder sleep and force the patients to move their legs or to walk around in the bedroom The sensations then disappear quickly As a rule there is no pain A few patients however have real pain with the same localization as the creeping sensations most often between the knee and ankle It is a constant slight to moderate aching pain which may appear alone or combined with creeping sensations It is disagreeable but not of great intensity The creeping sensa tion or pain in restless legs is as a rule felt on both sides symmetrically The grow ing pains in my cases were seldom felt in both legs at the same time The intensity of the ache seems to be much greater than in restless legs Both conditions have a predilection for the first hours of the night

Restless legs usually appear night after night but growing pains with greater inter vals Restless legs are relieved by diazepam growing pains by acetylsalicylic acid Grow ing pains diminish and disappear when the *children grow up* Restless legs may last for decades

I have seen a few adults with onset of restless legs in their childhood This is unusual The wrong diagnosis of growing pains had been made in these cases

The cause of restless legs and growing pains is still obscure Heredity seems to be important in both conditions No strict ge netic investigation has been made however Restless legs are made worse by *pregnancy* iron deficiency and some drugs (prometha zine) They disappear during fever Growing pains are not caused by growth but nothing is known about their origin

Acta Pædiatr Scand 64 264–266, 1975

GROWING PAINS AND RESTLESS LEGS

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ABSTRACT Ekbom K A (Department of Neurology University Hospital Uppsala Sweden) Growing pains and restless legs Acta Paediatr Scand 64 264 1975 —Growing pains is a common and distressing symptom in children but has aroused little interest There is a similarity between growing pains and the painful form of restless legs but it is not known if these two conditions are identical I have recently observed a family in which the mother has growing pains since her childhood The pain persists in adult age which is unusual She also has typical restless legs Her three sons have severe growing pains The study of this family has convinced me that growing pains and restless legs are different conditions

KEY WORDS Growing pains restless legs Ekbom s syndrome

Growing pains in children are common and cause much suffering Little has been written on this subject however For a review of the literature I refer to Øster (5) Growing pains and restless legs (Ekbom s syndrome) resemble each other Wersall (4) and Brenning (1) considered that the two conditions are identical or related I discussed this question in my monograph on restless legs (2) but did not come to any definite conclusion Twenty five years later I was still in doubt I then wrote that growing pains resembled the painful form of restless legs but added that the intensity of pain is probably greater in growing pains (3)

Most patients with restless legs have only disagreeable creeping sensations in their legs but some have real pain No pathological signs are found in either disease

I have seen several hundred patients with restless legs but until recently no adult with growing pains I have now however observed a woman who has suffered from growing pains since her childhood She also has restless legs and so has been able to compare the two conditions

CASE REPORTS

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A married schoolteacher 37 years old has for several years had periods of creeping sensations in her legs between the knee and ankle There is no pain The discomfort usually appears an hour after she has gone to bed and wakes her up from sleep She must then move her legs but does not leave the bed After half an hour or at most one hour she falls asleep again The creeping sensations have never appeared during the day or the evening but only when she is in bed They are provoked by exertion during the day Two years ago she went on a cycling tour during her holiday Then she had creeping sensations at night but they ceased when the tour was over

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Acta Pædiatr Scand 64 267–272 1975

CHEMICALLY DEFINED DIET IN THE TREATMENT OF KWASHIORKOR

DEMISSIE HABTE BENGT PERSSON and GÖRAN STERKY

From the Department of Paediatrics Haile Selassie I University Addis Ababa Ethiopia and the Department of Paediatrics St Goran s Hospital Stockholm Sweden

ABSTRACT Habte D Persson B and Sterky G (Department of Paediatrics Haile Selassie I University Addis Ababa Ethiopia and S t Goran s Hospital Stockholm Sweden) Chemically defined diet in the treatment of kwashiorkor Acta Paediatr Scand 64 267 1975.—Kwashiorkor is associated with malabsorption of energy and nutrients Standard diets often initiate diarrhea and a high mortality is still prevalent A synthetic monomolecular formula has been evaluated and compared with a standard diet in the early rehabilitation phase of 21 children with kwashiorkor The formula was well accepted by the children and initiated a satisfactory clinical response The formula group had significantly less vomiting and reached minimum weight faster than the group on standard diet Weight gain and diarrhea were similar The rise of albumin and BUN was faster on standard diet A significant increase in haemoglobin was seen only in the formula group A rise in body temperature after a meal was evident in most patients and significantly more pronounced in the formula group The lower total nitrogen content of the formula may explain the observed slower rise in albumin and BUN but the ready utilization was indicated by the favourable weight changes as well as the rise in rectal temperature As high energy per volume was desirable the formula was not diluted to isoosmolal ty However the high glucose concentration in the experimental diet probably caused some negative effects

KEY WORDS Kwashiorkor chemically defined diet

A significant obstacle to dietary rehabilitation of children with kwashiorkor is the extensive damage to intestinal structure usually associated with it and leading to a malabsorption state (17) In addition to mucosal atrophy of both the small and large intestines (8 10 25 30 32) pancreatic atrophy is a constant feature (4 11) The functional counterparts of these changes are malabsorption of carbohydrates (5 18) fat (13 23) protein (21) and water (25) Diminished activities of intestinal mucosal dipeptide hydrolase enzymes necessary for cleavage of dipeptides into amino acids has been reported and the possibility that this may result in reduced availability of amino acids and consequent growth retardation has been suggested (21) On the other hand impairment of absorption of exogenous amino acids has not been demonstrated in severe protein calorie malnutrition (34) although there is evidence to suggest that a continuous loss of nitrogenous substances of endogenous origin occurs in diarrhea that can result in impaired synthesis of intestinal mucosa and digestive enzymes (12)

Standard diets in use derive proteins from cow s milk and energy from vegetable fat and

Supported by grants from the Swedish Nutrition Foundation the Semper Fund for Nutritional Research and the Swedish Medical Research Council project no 19P-4106

In my opinion growing pains and restless legs are two different conditions. In clinical work the first step is always a correct diagnosis. An effective therapy based on an understanding of the pathogenesis often has to wait for many years (as for instance in pernicious anemia). Much research remains to be done concerning the two conditions discussed in this paper.

REFERENCES

1. Brenning, R.: Growing pains. *Acta Soc Med Upsal 65*: 185, 1960.
2. Ekbom, K. A.: Restless legs. *Acta Med Scand* Suppl *158*: 1, 1945.
3. Ekbom, K. A.: Restless legs. *In* P. J. Vinken & G. W. Bruyn (eds.), *Handbook of clinical neurology*, North Holland Publishing Company, Amsterdam 1970, vol. 8, part II, pp. 311–320.
4. Wersäll, J.: Restless legs. *Läkartidningen 49*: 2032, 1952.
5. Øster, J.: Growing pains. A symptom and its significance. *Dan Med Bull 19*: 72, 1972.

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Table 1 *Clinical data on 21 children with protein energy malnutrition (PEM)*

Mean values and within brackets ranges

	All patients	Surviving patients Vivonex	Casilan
Number of patients	21	9	6
Type of PEM			
kwashiorkor	14	7	4
marasmic kwashiorkor	7	2	2
Age months	25 1 (14–45)	24 6 (14–36)	26 3 (14–45)
Weight kg (on admission)	8 2 (5 7–12 7)	8 2 (6 6–9 5)	8 7 (5 7–12 7)
Days to attain minimum weight	-	7 1	14 0
Weight gain g/day (first 22 days)	-	38 2	36 3
Days in hospital		38 6	45 3

carbohydrates (lactose sucrose starch) It is a common clinical experience that diarrhea is frequently initiated by these diets To overcome this complication but still manage the treatment by the oral route protein hydrolysate supplements have been tried (16 24 28) The conflicting results reported and the recent availability of a fully synthetic chemically defined diet led us to investigate its value in the treatment of kwashiorkor As most of the defects in digestion–absorption encountered are transitory such a diet needs to be administered only for a limited period (4 9 11 19 25)

This paper reports on the clinical and routine biochemical evaluation of a synthetic feeding mixture with an almost exclusive monomolecular composition (22) in the rehabilitation of severely malnourished children [1] An account on the effect on lipid metabolism will be given in a separate communication

MATERIAL AND METHODS

Twenty one children with clinical features of kwashiorkor (20) referred from the out patient department of the Ethio-Swedish Pediatric Clinic were studied The majority of patients with protein-energy malnutrition were treated on an ambulatory basis in a nutrition rehabilitation clinic and thus the group under study represents only the severe form However patients with manifest life threatening infection already on admission were excluded On admission all patients were assessed by history physical examination routine analyses of blood urine and stool PPD skin test and chest X ray Some clinical data are summarized in Table 1

Patients were assigned to one of two dietary groups one group consisting of 13 patients receiving a synthetic mixture Vivonex 100 and a second group of 8 patients receiving a standard diet with a cow milk protein concentrate Casilan (Glaxo England) in routine use for dietary treatment of kwashiorkor Vivonex was provided as a powder which dissolved instantly in warm water and formed a clear solution Artificial flavour and colour were also added to it The standard diet contained besides Casilan skim milk powder vegetable oil and sugar The composition of the reconstituted mixtures of Vivonex and Casilan are shown in Table 2

Patients were admitted to a metabolic ward where the room temperature was maintained between 23 and 28°C using electric fan heaters Routine care provided for the patients included frequent observations of pulse respiration rate and rectal temperature administration of antibiotics (usually chloramphenicol 50 mg/kg/day unless specific pathogens were isolated or strongly suspected when appropriate antibiotics were substituted) iron and folic acid Feeds were given every 3rd hour in a volume of 100 ml/kg/day at the beginning but later increased on demand and were usually administered via a nasal gastric tube during the first few days Tea with sugar was given to all patients on the day of admission half strength Vivonex or Casilan on the 2nd day and full strength on the 3rd day This was continued for 3 weeks at which time whole cow s milk was introduced Semi solid foods were usually offered after 2 weeks of hospital stay

The effects of the diets on postprandial body temperature were determined during the first week after the 8 o clock morning feed Rectal temperature was measured before feeds and at 1 2 and 3 hours subsequently using a low reading thermometer

Biochemical Analysis

Venous blood (3 0 ml) was collected in Ellerman tubes An aliquot (1 8 ml) was transferred to a heparinized tube and kept in iced water until centrifugation Aliquots of plasma

Table 2 *Composition of diets (per 100 ml of mixture given)*

	Vivonex 100	Casilan
Energy kcal	100	98
Protein g	2 1 (aminoacids)	4 4 (casein)
Fat g	0 07	6 0
Carbohydrates g	22 2 (glucose)	7 0 (sucrose lactose)

[1] A preliminary report was presented at the Second International Symposium on Balanced Nutrition and Therapy Erlangen Germany April 1973

Table 3 *Changes in rectal temperature at 0 1 2 and 3 hours following a meal at 8 o clock during treatment of PEM*

Values are given as mean±S E M

Time hours	Day 2	p	Day 5	p	Day 8	p
Vivonex n=9						
0	37 1±0 16		37 0±0 20		37 3±0 08	
1	37 5±0 14	<0 05	37 4±0 16	<0 05	37 6±0 19	ns
2	37 6±0 14	<0 05	37 9±0 13	<0 001	37 5±0 11	ns
3	37 7±0 14	<0 01	37 5±0 19	<0 05	37 8±0 15	<0 05
Casilan n=6						
0	37 1±0 24		37 3±0 26		37 3+0 08	
1	37 3±0 10	ns	37 5±0 2?	<0 05	37 4±0 13	ns
?	37 4±0 13	ns	37 6±0 17	ns	37 5±0 2?	ns
3	37 4±0 23	ns	37 5±0 23	ns	37 4±0 34	ns

p indicates signifi ant differences (paired t test) from 0-values

were taken for determination of blood urea nitrogen BUN (?7) and glucose (hexokinase Boehringer) Serum was kept at +4°C and used for analysis of albumin (cellulose acetate electrophoresis Beckman Spinco FB 291) and total pro tein by the biuret technique Hemoglobin was determined on capillary blood using the cyanmethemoglobin method All analyses were done in duplicate Venous blood samples were drawn on the 2nd day of admission and every second day during the first week thereafter weekly until dis charge

RESULTS

Clinical

There were 6 deaths in the total material of which 4 were in the Vivonex and 2 in the Casilan groups Four out of 6 deaths occurred within the first week and were associated with diarrhea and dehydration in 4 hypoglycemia in 1 and congestive heart failure in another The high mortality corresponds to that observed among in patients in the hospital (31)

Vivonex was well accepted by all patients Although it is a practice to feed patients with kwashiorkor through a nasal gastric tube be cause of anorexia almost all of the patients took Vivonex from the bottle by the 2nd and 3rd hospital day in contrast to the group taking Casilan who usually needed to be tubefed for about a week Clinically there was a progres sive improvement among survivors with gen eral well being euphoria and return of appetite but the speed with which these set in was faster in the Vivonex group

There was often a slight increase in body weight during the 2nd and 3rd day which was followed by a rapid diuresis and attainment of minimum weight The Vivonex group reached minimum weight significantly faster ($p<0\ 05$) than the Casilan group (Table 1) The subse quent rate of gain in body weight calculated for the duration of the experimental diet was the same for both groups (Table 1)

There was no difference between the two dietary groups in the frequency of stool passed Two patients in each group needed intravenous therapy to correct dehydration resulting from diarrhea The frequency of vomiting was significantly higher ($p<0\ 001$ on day 3) among patients taking the Casilan diet

Table 3 summarizes change in rectal temper ature following a meal on representative days during the first week A rise in body tempera ture was evident in most patients This rise was more pronounced and significant during the first half of the week in patients taking Viv onex

Biochemical

The 21 patients had the following mean values Hemoglobin 10 3 g/100 ml albumin 1 67 g/100 ml blood urea nitrogen 6 5 mg/100 ml and glucose 86 4 mg/100 ml An exception to those low values was observed in 2 patients with

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Days in hospital		38 6	45 3

carbohydrates (lactose sucrose starch) It is a common clinical experience that diarrhea is *frequently* initiated by these diets To overcome this complication but still manage the treatment by the oral route protein hydrolysate supplements have been tried (16 24 28) The conflicting results reported and the recent availability of a fully synthetic chemically defined diet led us to investigate its value in the treatment of kwashiorkor As most of the defects in digestion–absorption encountered are transitory such a diet needs to be administered only for a limited period (4 9 11 19 25)

This paper reports on the clinical and routine biochemical evaluation of a synthetic feeding mixture with an almost exclusive monomolecular composition (22) in the rehabilitation of severely malnourished children [1] An account on the effect on lipid metabolism will be given in a separate communication

MATERIAL AND METHODS

Twenty one children with clinical features of kwashiorkor (20) referred from the out patient department of the Ethio-Swedish Pediatric Clinic were studied The majority of patients with protein energy malnutrition were treated on an ambulatory basis in a nutrition rehabilitation clinic and thus the group under study represents only the severe form However patients with manifest life threatening infection already on admission were excluded On admission all patients were assessed by history physical examination routine analyses of blood urine and stool PPD skin test and chest X ray Some clinical data are summarized in Table 1

Patients were assigned to one of two dietary groups one group consisting of 13 patients receiving a synthetic mixture Vivonex 100 and a second group of 8 patients receiving a standard diet with a cow milk protein concentrate Casilan (Glaxo England) in routine use for dietary treatment of kwashiorkor Vivonex was provided as a powder which dissolved instantly in warm water and formed a clear solution Artificial flavour and colour were also added to it The standard diet contained besides Casilan skim milk powder vegetable oil and sugar The composition of the reconstituted mixtures of Vivonex and Casilan are shown in Table 2

Patients were admitted to a metabolic ward where the room temperature was maintained between 23 and 25°C using electric fan heaters Routine care provided for the patients included frequent observations of pulse respiration rate and rectal temperature administration of antibiotics (usually chloramphenicol 50 mg/kg/day unless specific pathogens were isolated or strongly suspected when appropriate antibiotics were substituted) iron and folic acid Feeds were given every 3rd hour in a volume of 100 ml/kg/day at the beginning but later increased on demand and were usually administered via a nasal gastric tube during the first few days Tea with sugar was given to all patients on the day of admission half strength Vivonex or Casilan on the 2nd day and full strength on the 3rd day *This was continued for* 3 weeks at which time whole cow s milk was introduced Semi solid foods were usually offered after 2 weeks of hospital stay

The effects of the diets on postprandial body temperature were determined during the first week after the 8 o clock morning feed Rectal temperature was measured before feeds and at 1 2 and 3 hours subsequently using a low reading thermometer

Biochemical Analysis

Venous blood (3 0 *ml*) was *collected* in Ellerman tubes An aliquot (1 8 ml) was transferred to a heparinized tube and kept in iced water until centrifugation *Aliquots of plasma*

Table 2 *Composition of diets (per 100 ml of mixture given)*

	Vivonex 100	Casilan
Energy kcal	100	98
Protein g	2 1 (aminoacids)	4 4 (casein)
Fat g	0 07	6 0
Carbohydrates g	22 2 (glucose)	7 0 (sucrose lactose)

[1] A preliminary report was presented at the Second International Symposium on Balanced Nutrition and Therapy Erlangen Germany April 1973

by the high glucose concentration and the high osmotic load In a study of carbohydrate absorption in Ethiopian children with kwashiorkor using oral sugar tolerance tests glucose malabsorption was found in 41% of the patients (15) Furthermore a lumen concentration of glucose greater than 2 5% has been found to result in net loss of water into the gut (18) The high concentration of glucose (22%) in the formula must certainly contribute to the diarrhea seen in some of the patients and would appear to negate the advantages of complete absorption of other nutrients The dilution of Vivonex as used in this study resulted in a hyperosmolar solution and diarrhea caused by this high osmotic load has been known to occur following its administration (22)

A significant increase in mean hemoglobin concentration was seen only amongst the group receiving Vivonex The presence of trace metals in the chemical diet may be a factor accounting for this difference

A rise in body temperature following a meal has been found in children with kwashiorkor during the rapid growth that accompanies recovery (6 7) The nature and importance of dietary thermogenesis in normal man is still unclear (1 2) but during rehabilitation of infants with protein calorie malnutrition a rise of metabolic rate following the ingestion of food would be advantageous (6 7) The more significant rise in rectal temperature during the first few days amongst patients on Vivonex may well reflect better absorption and ready utilization of nutrients

The discrepancy between the two dietary groups with respect to serum albumin levels and the presence of oedema is a *paradoxical* finding Thus patients on Vivonex lost oedema faster and at a lower serum albumin concentration than those receiving Casilan The frequency of oedema is known to be variable for serum albumin concentrations below 3 5 g% (33) The mechanism of oedema formation in kwashiorkor is as yet incompletely understood although a number of factors are suspected to operate (29) Our results indicate that the serum albumin concentration does not appear to be the decisive factor during dietary rehabilitation

Kwashiorkor continues to have a high mortality even in children s hospitals Although the final goal is to prevent its development and/or to institute early dietary rehabilitation it will take long time before this is achieved A key factor for the high mortality is the unavailability of a high energy high protein diet that is easy to digest and absorb Our results indicate that a chemically defined diet consisting of elemental nutrients holds great promise for dietary rehabilitation of kwashiorkor but that the composition of such a diet should be carefully formulated to the needs of such patients A dilution of Vivonex 100 to iso-osmolality would result in too low energy per volume and restrict its value An alternative source of energy such as high molecular dextrines or medium chain triglycerides (14) seems necessary

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REFFRENCES

1 Ablett J G & McCance R A Energy expenditure of children with kwashiorkor *Lancet II* 517 1971
2 Ashworth A Malnutrition and metabolic rates *Nutr Rev 28* 279 1970
3 Ashworth A Bell R James W P T & Waterlow J C Caloric requirements of children recovering from protein-calorie malnutrition *Lancet I* 600 1968
4 Barbezat G O & Hansen J D L The exocrine pancreas and protein calorie malnutrition *Pediatrics 42* 77 1968
5 Bowie M D Barbezat G O & Hansen J D L Carbohydrate absorption in malnourished children *Am J Clin Nutr 20* 89 1967
6 Brooke O G Influence of malnutrition on the body temperature of children *Br Med J I* 331 1972
7 Brooke O G & Ashworth A The influence of malnutrition on the postprandial metabolic rate and respiratory quotient *Br J Nutr 27* 407 1972
8 Burman D The jejunal mucosa in kwashiorkor *Arch Dis Child 40* 5 6 1965

Table 4 *Haemoglobin albumin blood urea nitrogen and glucose during treatment of 15 surviving patients with PEM*

Values are expressed as means and S E M

	Vivonex ($n=9$)			Casilan ($n=6$)	
	Mean	S E M	p	Mean	S E M
Day 1					
Hb 3 g/100 ml	9 9	0 4	n s	9 8	0 8
Albumin g/100 ml	1 7	0 1	n s	1 6	0 2
BUN mg/100 ml	5 0	0 9	n s	7 0	1 2
Glucose mg/100 ml	100 6	18 5	n s	79 5	0 6
Day 8					
Hb g/100 ml	9 6	0 2	n s	9 5	0 6
Albumin g/100 ml	2 0	0 1	<0 01	3 0	0 4
BUN mg/100 ml	5 3	0 4	<0 001	10 7	0 9
Glucose mg/100 ml	76 6	8 8	n s	72 5	2 5
Day 15					
Hb g/100 ml	10 3	0 4	n s	9 3	0 6
Albumin g/100 ml	2 8	0 2	<0 01	3 7	0 1
BUN mg/100 ml	8 6	1 6	n s	12 4	1 2
Glucose mg/100 ml	76 2	3 4	n s	74 5	4 5
Day 22					
Hb g/100 ml	11 2	0 2	n s	10 1	0 8
Albumin g/100 ml	3 5	0 2	n s	3 7	0 3
BUN mg/100 ml	16 5	2 2	n s	11 1	1 6
Glucose mg/100 ml	81 3	0 6	n s	76 0	5 0

* p indicates significant differences between mean values of the Vivonex and Casilan groups n s =not significant

fasting blood glucose concentrations of 127 and 237 mg/100 ml respectively

During rehabilitation (Values given refer to surviving patients) Table 4 shows a progressive rise of albumin and BUN in both groups This rise attained statistical significance already by day 8 for the Casilan group ($p<0\ 01$) and by day 15 for Vivonex group ($p<0\ 01$) The Casilan group also had significantly higher mean values of albumin at day 8 and 15 as compared with those of the Vivonex group These changes were also paralleled by significant differences in BUN between the groups during the first 2 weeks A significant increase in mean haemoglobin concentration during the first 3 weeks was only seen in the Vivonex groups ($p<0\ 05$)

DISCUSSION

Hansen et al reported in 1956 that a synthetic amino acid mixture with glucose minerals and vitamins could initiate cure and positive nitrogen balance in patients with kwashiorkor (16) They concluded that amino acids are the chief limiting nutrients of the diets of which kwashiorkor develops Subsequently Senecal (28) reported highly beneficial effects from the use of protein hydrolysates but Pretorius & de Villiers (24) were unable to confirm this They found that the use of a protein hydrolysate supplement had no detectable advantage over the use of an equivalent supplement of casein

The present study has demonstrated that a complete chemically defined diet (Vivonex 100) made up of L aminoacids glucose essential fatty acids eletrolytes and multivitamins was well accepted and succeeded in reversing the acute manifestation of kwashiorkor In spite of its low nitrogen content minimum weight was attained in a shorter period of time and subsequent weight gain was as good as in patients treated with twice the amount of protein This possibility indicates the ready availability of nitrogen in Vivonex and also underlines the importance of the total caloric intake The rate of weight gain in children recovering from malnutrition has been shown to be significantly more influenced by the level of caloric intake than by that of protein (3 26) However the significantly slower rise in serum albumin and BUN in the Vivonex group reflects the lower total nitrogen intake

The failure of Vivonex to reduce the incidence of diarrhea associated with dietary rehabilitation of kwashiorkor might be explained

by the high glucose concentration and the high osmotic load In a study of carbohydrate absorption in Ethiopian children with kwashiorkor using oral sugar tolerance tests glucose malabsorption was found in 41% of the patients (15) Furthermore a lumen concentration of glucose greater than 2 5% has been found to result in net loss of water into the gut (18) The high concentration of glucose (22%) in the formula must certainly contribute to the diarrhea seen in some of the patients and would appear to negate the advantages of complete absorption of other nutrients The dilution of Vivonex as used in this study resulted in a hyperosmolar solution and diarrhea caused by this high osmotic load has been known to occur following its administration (22)

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The discrepancy between the two dietary groups with respect to serum albumin levels and the presence of oedema is a paradoxical finding Thus patients on Vivonex lost oedema faster and at a lower serum albumin concentration than those receiving Casilan The frequency of oedema is known to be variable for serum albumin concentrations below 3 5 g% (33) The mechanism of oedema formation in kwashiorkor is as yet incompletely understood although a number of factors are suspected to operate (29) Our results indicate that the serum albumin concentration does not appear to be the decisive factor during dietary rehabilitation

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4 Barbezat G O & Hansen J D L The exocrine pancreas and protein calorie malnutrition *Pediatrics 4?* 77 1968
5 Bowie M D Barbezat G O & Hansen J D L Carbohydrate absorption in malnourished children *Am J Clin Nut 20* 89 1967
6 Brooke O G Influence of malnutrition on the body temperature of children *B Med J I* 331 197?
7 Brooke O G & Ashworth A The influence of malnutrition on the postprandial metabolic rate and respiratory quotient *Br J Nut 27* 407 197
8 Burman D The jejunal mucosa in kwashiorkor *Arch Dis Child 40* 5 6 1965

9 Cook G C & Lee F D The jejunum after kwashiorkor *Lancet II* 1263 1966
10 Davies J N P The essential pathology of kwashiorkor *Lancet I* 317 1948
11 Danus O Urbina A M Valenzuela I & Solimano G The effect of refeeding on pancreatic exocrine function in marasmic infants *J Pediatr 77* 334 1970
12 Ghadimi H Kumar S & Abaci F Endogenous amino acid loss and its significance in infantile diarrhea *Pediatr Res 7* 161 1973
13 Gomez F Galvan R Cravioto J Frenk S Sanatella J & Pena C Fat absorption in chronic and severe malnutrition in children *Lancet II* 121 1956
14 Graham G G Baertl J M Cordano A & Norvales E Lactose free medium-chain triglyceride formulas in severe malnutrition *Am J Dis Child 126* 330 1973
15 Habte D Hyvarinen A & Sterky G Carbohydrate malabsorption in kwashiorkor *Ethiop Med J 11* 33 1973
16 Hansen J D L Howe E E & Brock J F Aminoacids and kwasiorkor *Lancet II* 911 1956
17 Herskovic T Protein malnutrition and the small intestine *Am J Clin Nutr 22* 300 1969
18 James W P T Sugar absorption and intestinal motility in children when malnourished and after treatment *Clin Sci 39* 305 1970
19 — Jejunal disaccharidase activities in children with marasmus and kwasiorkor Response to treatment *Arch Dis Child 46* 218 1971
20 Jelliffe D B Infant nutrition in the subtropics and tropics World Health Organization Monograph Series No 53 Geneva 1966
21 Kumar V Ghai O P & Chase H P Intestinal dipeptide hydrolase activities in undernourished children *Arch Dis Child 46* 801 1971
22 Lang K Fekl W & Berg G (Eds) *Balanced nutrition and therapy International Symposium in Nuremberg* April 10–12 1970 George Thieme Verlag Stuttgart 1971
23 Oliveira J E D & Rolando E Fat absorption studies in malnourished children *Am J Clin Nutr 15* 287 1964
24 Pretorius P J & de Villiers L S The effect of protein hydrolysate on initiation of cure and various biochemical indices of recovery in kwashiorkor *S Afr J Lab Clin Med 7* 108 1961
25 Redmond A O B Kaschula R O C Freeseman C & Hansen J D L The colon in kwashiorkor *Arch Dis Child 46* 252 1968
26 Rutishausen I H E & McCance R A Calorie requirements for growth after severe undernutrition *Arch Dis Child 43* 25? 1968
27 Searcy R L Simms N M & Foreman J A A study of the specificity of the Berthelot colour reaction *Clin Chim Acta 12* 170 1965
28 Sénécal J The treatment and prevention of kwashiorkor in French West Africa *Ann Ny Acad Sci* 69 916 1958
29 Srikantia S G The causes of oedema in protein calorie malnutrition In *Calorie deficiencies and protein deficiencies* (ed R A McCance and E M Widdowson) J & A Churchill Ltd London 1968
30 Stanfield J P Hutt M S R & Tunnicliffe R Intestinal biopsy in kwashiorkor *Lancet II* 519 1965
31 Tafesse B Analysis of admissions of the Ethio-Swedish Paediatric Clinic (1970–71) *Ethiop Med J 11* 3 1973
32 Trowell H C Davies J N P & Dean R F A *Kwashiorkor* Edward Arnold Ltd London 1954
33 Whitehead R G Frood J D L & Poskitt E M E Value of serum albumin measurements in nutritional surveys A reappraisal *Lancet II* 287 1971
34 Wood Walker R B Hansen J D L & Saunders S J Jejunal absorption of amino acids in nutritional rickets *Lancet II* 1428 1969

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CONGENITAL GENERALIZED LIPODYSTROPHY

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ABSTRACT Najjar S Salem G and Idriss Z (Department of Pediatrics The American University of Beirut Beirut Lebanon) Congenital generalized lipodystrophy Acta Paediatr Scand 64 273 1975.—Six patients with congenital generalized lipodystrophy are described They had generalized paucity of fat tissue acanthosis nigricans prominent superficial veins and muscle hypertrophy They were mentally retarded Three had corneal opacities They had normal external genitalia and none was tall for age Their bone age was advanced and some had minor skeletal anomalies and nephromegaly The muscle histology on light microscopy was normal The majority had elevated serum aldolase and to a lesser degree serum lactic dehydrogenase and creatinine phosphokinase Four of five examined had a myopathic electromyogram They had normal or minimally deranged liver function tests The fatty liver infiltration in one seems to be progressive Four had a normal and two an abnormal metyrapone test They had an age-dependent abnormality of growth hormone insulin and carbohydrate homeostasis

KEY WORDS Lipodystrophy muscles growth hormone insulin glucose tolerance

Congenital Generalized Lipodystrophy (CGL) is a rare disorder of unknown etiology and pathogenesis Recently Seip published a comprehensive and detailed review of the subject (15) He collected 42 cases of CGL published until 1971 in the world literature including his 6 cases and offered convincing evidence for separating the congenital from the acquired form of Generalized Lipodystrophy

In the past 10 years we have encountered 6 cases of CGL at the American University Hospital The purpose of this communication is to present the salient clinical and biochemical features of these cases with emphasis on endocrine function and carbohydrate metabolism

MATERIAL

Cl c l feat es

There were 4 boys (J S Y S R I and B B) and 2 girls (F T and N T) from 4 unrelated families the two girls were sisters and two of the boys were brothers (J S and Y S) In 3 families the parents were first cousins (families S T and B) there was no consanguinity in family I A sister of B B was reported to have had similar physical appearance she died at an early age The paucity of subcutaneous fat was evident at birth or at least recognized retrospectively by the parents to have been present very early in infancy in all the children The age at which they were first seen varied from 5 months to 14 years There was striking resemblance particularly of the facies among the six as illustrated in Fig 1 None was tall for age using the norms of the Iowa Growth Chart They all had the characteristic generalized loss of subcutaneous fat including the fat pads of the cheeks The superficial veins of the extremities were prominent Five had hypertrichosis which was pronounced in four and minimal in one Acanthosis nigricans was present in all Four had prominent muscles All had normal external genitals Five had hepatomegaly and one had splenomegaly Corneal opacities were found in 3 of the five examined and consisted of punctate subepithelial deposits which were in band form in the lower one third of the cornea in two and in the center of the cornea of the right eye in one The full scale IQ performed on three ranged from 64 to 66 R I at two and a half years of age could not be tested adequately but it was the impression of the psychologist that the boy was retarded Two had no formal IQ testing but were mentally retarded

9 Cook G C & Lee F D The jejunum after kwashiorkor *Lancet II* 1763 1966
10 Davies J N P The essential pathology of kwashiorkor *Lancet I* 317 1948
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Histologic studies

Muscle and sural nerve biopsies were performed on five (F T N T Y S J S and R I) On light microscopy there were no signifi ant histologic changes in the nerves or muscles Percutaneous liver biopsies were performed on R I and F T R I had two liver biopsies one at 5 months of age and the other at the age of 2½ years F T at the age of 8 years had micronodular cirrhosis of the liver The liver of R I showed minimal to moderate fat infiltration at 5 months that became massive at the age of 2½ years Skin biopsy was successful in four (F T J S Y S and R I) It showed poorly formed fat lobules fibrous lobulation of the subcutaneous tissue and a marked diminution in fat tissue the latter finding however was not pronounced in the biopsy of R I when performed at the age of 5 months

Miscellaneous tests

Chromosomal analyses were performed on five F T N T Y S J S and R I All had a normal karyotype with 46 chromosomes and a normal sex chromosome complement

Five (F T N T Y S J S and R I) had electromyographic examination of three muscles the antenor tibial the gastrocnemius and the vastus lateralis Four had a myopathic pattern (marked reduction in amplitude polyphasic potentials and good interference) one R I had a normal electromyogram

Electrocardiographic tracings on F T Y S and R I were normal N T had voltage criteria for left ventricular hypertrophy One F T had an electroencephalogram which was interpreted as normal

METHODS

Blood for growth hormone (HGH) assay was obtained from an indwelling catheter at 20 minutes interval for one hour after the observed onset of sleep The stage of sleep however was not monitored neither by electroencephalogram nor by electromyogram to establish the onset of stage 3 or 4 (Non Rapid Eye Movement) sleep

Sequential Arginine Insulin Tolerance Test (AITT) (12) Arginine monochloride 0 5 g/kg body weight was administered intravenously over a period of 30 minutes and blood samples for glucose and HGH were obtained before and 30 45 and 60 minutes later at 60 minutes crystalline insulin (0 07 to 0 3 u/kg body weight) was administered intravenously in a single bolus and blood samples were taken 20 40 and 60 minutes later

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In none of the samples obtained from the 6 patients following the onset of sleep did the HGH concentration reach levels above 5 2 ng/ml

The results of the AITT are shown in Table 2 R I had an increase in the serum concentration of HGH in response to arginine and to insulin induced hypoglycemia at the age of 5 months but not when he was 2½ years old F T had an appreciable HGH release following insulin induced hypoglycemia at 2½ years of age (arginine was not given to her at this time) but not during the AITT at 8 years of age The other four had no HGH release during the AITT Adequate hypoglycemia however was achieved only in R I

Table 2 *Arginine Insulin tolerance tests*

Initials	F T				N T		I S		Y S		R I				B B	
Age performed (y)	?½		8		5?	5?	7		14		5/12		?½		64	
	G*	HGH	G	HGH	G	HGH	G	HGH	G	HGH	G	HGH	G	HGH	G	HGH
Time (min)																
0																
30	–	–	95	0	94	0 4	101	0	104	0 ?	–	1?	10?	3 0	–	3 6
45	–	–	11?	0 7	117	1 1	1 7	0	109	3 0	–	14 ?	104	1 4	–	3 4
60	–	–	115	1 1	105	1 7	1?5	0 6	109	6 6	–	11 ?	–	–	–	3 ?
80	91	0	96	9	86	0 7	99	0	109	4 4	80	19 8	106	2 0	100	4 0
100	58	7 0	79	0 4	77	0 7	88	0 ?	150	1 2	35	14 8	74	1 8	63	3 6
1 0	88	7 5	80	0	79	0 3	100	0	113	0 8	?0	5 0	48	1 8	98	5 4
	60	10 0	85	0 6	83	1 2	9	0	1.5	1 3	30	4 6	71	2 8	100	4 8

Glucose mg/100 ml
Growth Hormone ng/ml



Fig 1 Faces of the 6 patients From left to right Top F T N T and J S Bottom Y S R I and B B

All but one had normal hemoglobin and hematocrit concentration B B had a mild anemia The white blood count and differential were within normal limits in all They could concentrate their urine and none had pro teinuria glucosuria or formed elements in the urinary sediment The serum concentration of sodium potas sium chloride CO_2 combining power creatinine and urea nitrogen were normal in all 6 The levels of serum transaminases (SGOT and SGPT) were either normal or slightly elevated the serum albumin and globulin con centrations were normal in all but one Y S who had a serum albumin of 5 g per 100 ml Bromsulfophthalein (BSP) retention was normal in 5 but minimally increased (6% retention in 45 minutes) in one J S The serum concentration of creatinine phosphokinase (CPK) and lactic dehydrogenase (LDH) were either normal or slightly elevated Serum aldolase levels were normal in two (N T and F T) but moderately elevated in the rest (11 to 19 Sigma Units) The serum cholesterol levels were slightly elevated for age in two (215 mg/ 100 ml in R I and 213 mg/100 ml in Y S) while the plasma triglycerides were elevated in all minimally in B B (152 mg/100 ml) and to very high levels in N T (952 mg/100 ml) The fasting free fatty acid concentra tions in the serum were normal in 5 on whom they were determined They all had normal serum thyroxine con centration and four on whom serum TSH levels were assayed had normal values The plasma cortisol con centration determined by protein binding competitive assay on samples obtained between 8 and 9 a m the 24 hour urinary excretion of 17 hydroxycorticoids (17 O HC) and 17 ketosteroids and the response to the oral administration of metyrapone are shown in Table 1

Roentgenographic examination

The bone age was advanced in all The six had normal skull radiographs The intravenous pyelogram was nor mal in 4 F T and R I had evidence of right kidney enlargement None had any evidence of intrinsic bone disease Chest roentgenogram revealed a bifid manu brium in one (R I) and a bifid anterior end of the right fourth rib in another (Y S) it was normal in the other four All had radiologic findings suggesting absence of fat tissue None had sclerosis of the bone

Table 1 *Adrenal function tests*

Initials	AM Plasma cortisol μg/100 ml	17 KS mg/ 24 hr	Metyrapone[a] 17 OHC mg/24 hr		
			Day 1	Day 2	Day 3
F T	5 2	1 6	2 8	1 7	2 0
N T	5 0	2 8	1 1	3 6	2 9
J S	6 9	4 1	1 5	12 7	4 3
Y S	7 5	2 8	1 8	5 7	5 7
R I	9 25	0 41	2 5	6 0	3 4
B B	2 8	6 4	1 0	1 2	1 1

Normal values 5–10 μg/100 ml
[a] Metyrapone was administered orally in a dose of 250 mg/M2 body surface area every 4 hours for six doses 24 hour urine for 17 hydroxycorticoids was collected on the day prior to (Day 1) the day of (Day 2) and the day after (Day 3) the administration of metyrapone

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Muscle and sural nerve biopsies were performed on five (F T N T Y S J S and R I) On light micro copy there were no significant histologic changes in the nerves or muscles Percutaneous liver biopsies were performed on R I and F T R I had two liver biopsies one at 5 months of age and the other at the age of 7½ years F T at the age of 8 years had micronodular cirrhosis of the liver The liver of R I showed minimal to moderate fat infiltration at 5 months that became massive at the age of 2½ years Skin biopsy was suc cessful in four (F T J S Y S and R I) It showed poorly formed fat lobules fibrous lobulation of the sub cutaneous tissue and a marked diminution in fat tissue the latter finding however was not pronounced in the biopsy of R I when performed at the age of 5 months

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Initials	F T				N T		J S		Y S		R I				B B	
Age performed (y)	2½		8		5² 5–		7		14		5/12		2½		6½	
	G*	HGH	G	HGH	G	HGH	G	HGH	G	HGH	G	HGH	G	HGH	G	HGH
Time (min)																
0	–	–	95	0	94	0 4	101	0	104	0 ?	–	1	10?	3 0	–	3 6
30	–	–	11?	0 7	117	1 1	1?7	0	109	3 0	–	14 ?	104	1 4	–	3 4
45	–	–	115	1 1	105	1 7	125	0 6	109	6 6	–	11 ?	–	–	–	3 ?
60	91	0	96	? 9	86	0 7	99	0	109	4 4	80	19 8	106	? 0	100	4 0
80	58	7 0	79	0 4	77	0 7	88	0	150	1 2	35	14 8	74	1 8	63	3 6
100	88	7 5	80	0	79	0 3	100	0	113	0 8	70	5 0	48	1 8	98	5 4
1 0	60	10 0	85	0 6	83	1 ?	9?	0	1_5	1 3	30	4 6	71	? 8	100	4 8

Glucose mg/100 ml
Growth H[illegible]

Table 3 *Insulin tolerance tests expressed as mg glucose per 100 ml*

Initials	F T		N T		J S		Y S	R I		B B
Age performed (y)	2½	8	$5\frac{9}{12}$	$5\frac{9}{12}$	7	7	14	5/12	2½	6½
Insuline dose[b]	0 1	0 2	0 15	0 25	0 07	0 15	0 15	0 1	0 15	0 3
Time (min)										
0	91	96	86	94	99	112	122	80	106	100
20	58	79	77	90	89	92	110	35	74	63
40	88	80	79	74	100	104	95	20	48	90
60	90	85	73	79	92	106	85	30	71	100

[b] Units per kg of body weight

and in F T when 2½ years of age HGH levels did not change appreciably during the oral glucose tolerance tests they remained low

Carbohydrate metabolism

The results of the ITT are shown in Table 3 R I had an appreciable drop in the blood glucose level at the age of 5 months he had a response of a smaller magnitude at the age of 2½ years with a larger dose of insulin F T at 2½ years of age had a 30% drop in blood glucose level following the administration of 0 10 u/kg of insulin but no appreciable drop following 0 20 u/kg at the age of 8 years The other four had no hypoglycemia in response to relatively large doses of insulin (0 15 to 0 3 u/kg body weight)

The results of the OGTT are shown in Table 4 Only Y S the eldest of the six had glucose intolerance the rest had a normal or a flat response to the oral glucose load The plasma levels of IRI during the OGTT were normal in R I and B B and in F T when she was 2½ years of age The other 3 and F T at an older age had elevated plasma IRI levels Y S the eldest of the six and the most intolerant to oral glucose did not have the highest plasma IRI levels The concentration of FFA in the plasma were normal in the fasting state and suppressed normally following the administration of glucose

DISCUSSION

The clinical manifestations of our 6 patients were typically those of CGL They include a paucity of subcutaneous fat noted early in infancy hypertrichosis acanthosis nigricans prominent superficial veins prominent muscles hepatomegaly and mental retardation

Table 4 *Oral glucose tolerance tests*

Initials	F T				N T		J S		Y S		R I				B B	
Age performed (y)	2½		8		5^{9}		7		14		5/12		2½		6½	
	G[a]	IRI[b]	G	IRI	G	IRI	G	IRI	G	IRI	G	IRI	G	IRI	G	IRI
Time (min)																
0	112	22 5	97	65	95	56	100	106	140	40	81	18	75	27	90	36
30	112	56	178	268	110	127	121	320	212	72	124	17	96	42	122	76
60	105	56	128	196	101	214	123	320	237	106	114	78	98	32	60	56
120	111	37 5	107	230	104	214	105	246	230	136	117	23	110	47	95	50
180	99	35	99	140	75	54	91	110	138	60	81	12	90	30	74	30
240	–	–	76	50	75	36	88	64	100	33	–	–	–	–	79	23

[a] G Glucose mg/100 ml
[b] IRI Immunoreactive insulin mU/ml

Unlike most cases reported in the literature our patients were not tall for age this probably represents the inadequacy of the growth standards used for the particular socioeconomic background of these children Corneal opacities which have been described only by Seip in some of his patients (15) were present in 3 of 5 examined for this abnormality Clitoral or penile enlargement frequently reported in cases of CGL was not present in our cases The high incidence of consanguinity among the parents of our patients and the occurrence of the disease in siblings is in agreement with the current concept that CGL is a genetic disorder inherited as an autosomal recessive trait

The laboratory and roentgenographic findings were also in accord with those published in the literature Hypertriglyceridemia was the only persistent abnormality of blood lipids concentration Liver function tests were normal with few minor disturbances even in those with histologic evidence of massive fatty infiltration or cirrhosis Elevated levels of serum albumin have been reported in some cases of CGL (3) only one of our patients had an elevated serum albumin The basal metabolic rate which has frequently been reported to be elevated in cases of CGL was not determined in our patients None of our patients had sclerosis of the bones as described by Seip (15) who also found abnormal pneumoencephalograms in his 6 patients none of our subjects had air contrast studies of the ventricular system

Little besides the anatomic description is found in the literature relating to the skeletal muscles in CGL Detailed histologic description is lacking Senior (18) reported slight hypertrophy of the abdominal muscles of his patient Our cases had normal findings on microscopic examination of the skeletal muscles They also had normal sural nerve histology Some of them had mild to moderate increase in the serum enzymes thought to originate primarily from skeletal muscles Similar findings have been reported by Brunzell (3) It is possible that on higher magnification and resolution histologic abnormalities may become apparent because in contrast to the normal histologic findings and the minor serum enzymatic changes four of five of our patients had a definite myopathic pattern on electromyographic examination

Thyroid function studies in our patients and those reported by others (9 15 17) were normal Urinary excretion of 17 ketosteroids and 17 hydroxycorticoids in patients with CGL were reported as normal by some (3 4 13) or slightly elevated by others (1 17) Our patients had normal excretion of 17 hydroxycorticoids and only one had a modest increase in urinary 17 ketosteroids excretion Pituitary ACTH reserve was normal in 4 of 6 of our cases tested with metyrapone Variable responses to metyrapone have been reported in cases of CGL (1 13 17)

The basal levels of plasma HGH were normal or low in our cases as well as in those cases of CGL reported in the literature (4 7 9 14) Our patients had no HGH release following physiologic and pharmacologic stimuli except for R I and F T when 5 months and 2½ years old respectively but not at an older age Similar findings were obtained by Oseid (10) who studied the 6 cases of Seip The adequacy of the physiologic stimulus for HGH release used in our patients was not established Oseid (10) used exercise as a physiologic stimulus for HGH release in two of his patients they had no appreciable rise in plasma HGH None of our patients (except R I when 5 months old) and none of the patients of Oseid had HGH release following arginine administration Moreover the cases reported by Oseid had no HGH release following vasopressin administration The lack of HGH response to hypoglycemia in our patients and those of Oseid cannot be properly evaluated since adequate hypoglycemia (a fall of more than 40% in blood glucose level) was not achieved following insulin administration except in R I Oseid (10) however considered the

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Time (min)										
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fall in blood glucose of 20 to 30 mg% which occurred in 5 of his patients and an adequate stimulus for HGH release (5)

Gordon et al (6) reported a unique observation in a 2 years 7 months old girl with CGL who not only had elevated basal levels of HGH but had a paradoxical response to hyperglycemia and hypoglycemia This is of particular interest because of the roentgenographic abnormalities demonstrated in the hypothalamic area of patients with CGL (15) and the observed paradoxical HGH release reported in patients with organic lesions involving that area (2) None of our patients nor the patient reported by Kaplan (8) had paradoxical HGH release following oral glucose administration

The abnormality of HGH homeostasis may possibly be age dependent R I had a normal response to arginine and hypoglycemia when 5 months of age but not at the age of 2 years Similarly F T had a modest rise in plasma HGH following hypoglycemia at the age of 2½ years but not subsequently The 6 months old case reported by Fairney et al (4) also had a normal HGH response to prolonged fasting hypoglycemia and Bovril® it is not mentioned however whether he was restudied at a later age More cases need to be studied to confirm this observation

Oseid (10) discussed in detail the possible causes for the abnormal HGH homeostasis A disturbance in the hypothalamic pituitary axis seems to be the most likely

The insulin and carbohydrate abnormalities observed in our patients were similar to those described by Oseid (11) He studied his six cases at different ages from infancy to adolescence with regard to glucose tolerance insulin secretion and insulin sensitivity Early in infancy the only abnormality he found was some degree of insulin resistance With age insulin resistance became more marked glucose tolerance decreased and the serum insulin levels became grossly elevated One of his patients followed beyond the age of 12 years developed clinical diabetes mellitus and her serum insulin levels although elevated responded poorly to glucose and tolbutamide administration Most of our patients were studied only once but they exhibited similar age dependent changes in their carbohydrate and insulin homeostasis The high levels of circulating IRI in CGL have been shown to be primarily true insulin of pancreatic origin and of high biologic activity (19 20)

It appears that CGL is a progressive disease The fatty infiltration of the liver increases with age the cirrhotic changes are found in older children and the insulin resistance glucose intolerance elevation of plasma IRI and the abnormality in HGH homeostasis seem to be age dependent and are apparently absent in early infancy The pathogenesis of CGL however remains unknown The various theories advanced to explain the clinical and biochemical findings of this disease have been reviewed by Seip (15) He favors a diencephalic disturbance at least in most cases of CGL that may exert its influence either through a lipotropic hormone or via the autonomous nervous system rather than a primary abnormality of the adipose tissue

REFERENCES

1 Asano S Matsuki S Ozawa Y Saruta T & Nagashima H A case of Seip Lawrence syndrome with acanthosis nigricans *Keio J Med 15 101 1966*

2 Beck P Parker M L & Daughaday W H Paradoxical hypersecretion of growth hormone in response to glucose *J Clin Endocrinol Metab 26 1 463 1966*

3 Brunzell J D Shankle S W & Bethune J E Congenital generalized lipodystrophy accompanied by cystic angiomatosis *Ann Intern Med 69* 501 1968

4 Fairney A Lewis G & Cottom D Total lipodystrophy *Arch Dis Child 44* 368 1969

5 Glick S M Hypoglycemic threshold for human growth hormone release *J Clin Endocrinol Metab 36* 619 1970

6 Gordon H Pimstone B L Leary P M & Gordon W *Congenital generalized lipodystrophy* with abnormal growth hormone homeostasis *Arch Derm 104* 551 1971

7 Hamwi G J Kruger F A Eymontt M J Scarpelli D G Gwinup G & Byron R Lipoatrophic diabetes *Diabetes 15* 67 1966
8 Kaplan B S Congenital generalized lipodystrophy *S Afr Med J 44* 945 1970
9 Mabry C C Hollingsworth D R Upton V G & Corbin A Pituitary hypothalamic dysfunction in generalized lipodystrophy *J Pediatr 8?* 6?5 1973
10 Oseid S Studies in congenital generalized lipodystrophy III Growth hormone levels *Acta Endocrinol 73* 4?7 1973
11 — Studies in congenital generalized lipodystrophy I Development of diabetes *Acta Endocrinol 72* 475 1973
1? Penny R Blizzard R M & Davis W T Sequential arginine and insulin tolerance tests on the same day *J Clin Endocrinol Metab 29* 1499 1969
13 Ruvalcaba R H A & Kelley V C Lipodystrophic diabetes *Am J Dis Child 109* 287 1965
14 Samaan N A & Craig J W Serum insulin and growth hormone in lipoatrophic diabetes *Metabolism 18* 460 1969
15 Seip M Generalized lipodystrophy *Ergeb Inn Med Kinderheilkd 31* 59 1971
16 — Lipodystrophy and gigantism with associated endocrine manifestations A new syndrome? *Acta Paediatr Scand 48* 555 1959
17 Seip M & Trygstad O Generalized lipodystrophy *Arch Dis Childh 38* 447 1963
18 Senior B Lipodystrophic muscular hypertrophy *Arch Dis Childh 16* 4?6 1961
19 Søvik O & Oseid S Studies in congenital generalized lipodystrophy *Acta Endocrinol 72* 495 1973
?0 Søvik O Oseid S & Øyasæter S Studies in congenital generalized lipodystrophy V Circulating insulin and proinsulin *Acta Endocrinol 73* 731 1973

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The insulin and carbohydrate abnormalities observed in our patients were similar to those described by Oseid (11) He studied his six cases at different ages from infancy to adolescence with regard to glucose tolerance insulin secretion and insulin sensitivity Early in infancy the only abnormality he found was some degree of insulin resistance With age insulin resistance became more marked glucose tolerance decreased and the serum insulin levels became grossly elevated One of his patients followed beyond the age of 12 years developed clinical diabetes mellitus and her serum insulin levels although elevated responded poorly to glucose and tolbutamide administration Most of our patients were studied only once but they exhibited similar age dependent changes in their carbohydrate and insulin homeostasis The high levels of circulating IRI in CGL have been shown to be primarily true insulin of pancreatic origin and of high biologic activity (19 20)

It appears that CGL is a progressive disease The fatty infiltration of the liver increases with age the cirrhotic changes are found in older children and the insulin resistance glucose intolerance elevation of plasma IRI and the abnormality in HGH homeostasis seem to be age dependent and are apparently absent in early infancy The pathogenesis of CGL however remains unknown The various theories advanced to explain the clinical and biochemical findings of this disease have been reviewed by Seip (15) He favors a diencephalic disturbance at least in most cases of CGL that may exert its influence either through a lipotropic hormone or via the autonomous nervous system rather than a primary abnormality of the adipose tissue

REFERENCES

1 Asano S Matsuki S Ozawa Y Saruta T & Nagashima H A case of Seip Lawrence syndrome with acanthosis nigricans *Keio J Med 15* 101 1966

2 Beck P Parker M L & Daughaday W H Paradoxical hypersecretion of growth hormone in response to glucose *J Clin Endocrinol Metab 26* 463 1966

3 Brunzell J D Shankle S W & Bethune J E Congenital generalized lipodystrophy accompanied by cystic angiomatosis *Ann Intern Med 69* 50[illegible] 1968

4 Fairney A Lewis G & Cottom D Total lipodystrophy *Arch Dis Child 44* 368 1969

5 Glick S M Hypoglycemic threshold for human growth hormone release *J Clin Endocrinol Metab 36* 619 1970

6 Gordon H Pimstone B L Leary P M & Gordon W Congenital generalized lipodystrophy with abnormal growth hormone homeostasis *Arch Derm 104* 551 1971

Hamwi G J Kruger F A Eymontt M J Scarpelli D G Gwinup G & Byron R Lipoatrophic diabetes *Diabetes 15* 762 1966
Kaplan B S Congenital generalized lipodystrophy *S Afr Med J 44* 945 1970
Mabry C C Hollingsworth D R Upton V G & Corbin A Pituitary hypothalamic dysfunction in generalized lipodystrophy *J Pediatr 82* 625 1973
Oseid S Studies in congenital generalized lipodystrophy III Growth hormone levels *Acta Endocrinol 73* 477 1973
— Studies in congenital generalized lipodystrophy I Development of diabetes *Acta Endocrinol 72* 475 1973
Penny R Blizzard R M & Davis W T Sequential arginine and insulin tolerance tests on the same day *J Clin Endocrinol Metab 29* 1499 1969
Ruvalcaba R H A & Kelley V C Lipodystrophic diabetes *Am J Dis Child 109* 287 1965
Samaan N A & Craig J W Serum insulin and growth hormone in lipoatrophic diabetes *Metabolism 18* 460 1969
15 Seip M Generalized lipodystrophy *Ergeb Inn Med Kinderheilkd 31* 59 1971
16 — Lipodystrophy and gigantism with associated endocrine manifestations A new syndrome? *Acta Paediatr Scand 48* 555 1959
17 Seip M & Trygstad O Generalized lipodystrophy *Arch Dis Childh 38* 447 1963
18 Senior B Lipodystrophic muscular hypertrophy *Arch Dis Childh 16* 4 6 1961
19 Søvik O & Oseid S Studies in congenital generalized lipodystrophy *Acta Endocrinol 72* 495 1973
20 Søvik O Oseid S & Øyasæter S Studies in congenital generalized lipodystrophy V Circulating insulin and proinsulin *Acta Endocrinol 73* 731 1973

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4 Fairney A Lewis G & Cottom D Total lipodystrophy *Arch Dis Child 44* 368 1969
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		0*	max level	180	0	30	60	180	0	60	0	30	60
Asymptomatic (Group A)	7	81 ±13	179 ±25	80 ±11	10 9 ±5 7	23 9 ± 4 5	40 9 ±19 3	15 4 ±10 6	6 5 ±9 9	2 8 ±2 5	359 ±246	282 ±221	117 ± 82
Symptomatic transient (Group B)	8	63 ±14	171 ±45	78 ±24	11 0 ±6 3	57 0 ±44 2	59 0 ±75 9	13 5 ±12 3	5 0 ±5 6	2 3 ±2 0	509 ±145	271 ± 73	205 ±129
Secondary (Group C)	8	74 ± 6	135 ±24	78 ±14	8 1 ±7 0	37 5 ±16 6	51 7 ±30 0	10 7 ± 7 1	3 8 ±5 9	2 9 ±5 2	392 ±203	207 ± 73	96 ± 25
Controls	7	70 ±17	111 ±29	74 ±70	9 6 ±4 6	36 0 ± 9 0	25 8 ± 8 9	15 4 ± 6 2	2 8 ±2 6	2 2 ±2 1	235 ± 52	117 ± 18	72 ± 25

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A family history of juvenile diabetes mellitus was elicited in 2 patients from group C and in two of the controls

Two patients in group B (R D and I L) had intravenous tolbutamide test with a dose of 0 mg per kg body weight and oral leu ine tolerance test with 150 mg per kg body weight They had both experienced hypoglycaemic episodes after the neonatal period Patient I L had several convulsions and fasting blood glucose value of 18 mg/100 ml had been noted on one occasion after the newborn period At the present follow up examination the blood glucose level was 38 mg/100 ml after 24 hours starvation No hypoglycaemic symptoms were noted during this test The other patient R D also had several convulsions mainly occurring after limited food intake Fasting blood glucose level was 33 mg/100 ml prior to the glucose tolerance test He has spastic cerebral palsy and is mentally retarded

Blood glucose was measured by the ortho toluidine method (7 9) Free fatty acids (FFA) were determined colorimetrically as described by Duncombe (2)

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For statistical analyses the Students t test was applied

RESULTS

Among 37 children seen at the follow up study 2 patients from group B had experienced hypoglycaemic episodes after the neonatal period Another patient from group A who died at 10 weeks of age following an operation for incarcerated inguinal hernia showed terminal blood glucose values of 14 and 8 mg/100 ml No further information was available on this patient

The results of the oral glucose tolerance test are presented in Table 1 Blood glucose increment was 59% of the fasting level in group A 92% in group B and 83% in group C compared with 58% in controls Maximum insulin response was noted at 60 minutes in all categories of patients being most pronounced in group B In controls the peak insulin response occurred at 30 minutes The differences were not statistically significant except for insulin level at 30 minutes in group A which was significantly lower than in controls ($p<0\ 01$) Serum growth hormone and FFA level decreased during the first hour following glucose ingestion in all subjects showing essentially similar pattern in the different groups The FFA level at 0 and 30 minutes was significantly higher in group B than in controls and in group C at 30 minutes ($p<0\ 01$) There were no significant differences between the different patient groups

Three patients showed a diabetic blood glucose curve (Fig 1) The insulin response in these patients was considerably higher than in the controls and patient I L showed plasma insulin increment from 21 ng/ml in the fasting state to 255 ng/ml at 60 minutes Another pa

Acta Pædiatr Scand 64 280–286, 1975

ENDOCRINOLOGICAL ASPECTS AT FOLLOW UP STUDIES IN NEONATAL HYPOGLYCAEMIA

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From the Department of Paediatrics and the Hormone Laboratory University of Bergen Bergen Norway

ABSTRACT Fluge G Stoa K F and Aarskog D (Department of Paediatrics and Hormone Laboratory University of Bergen Bergen Norway) Endocrinological aspects at follow up studies in neonatal hypoglycaemia Acta Paediatr Scand 64 280 1975 —Thirty seven cases of neonatal hypoglycaemia were studied at follow up at the age of 2 6/12 – 4 9/12 years Two of them had had hypoglycaemia after the newborn period and another patient died in a hypoglycaemic state following surgery at 10 weeks of age Twenty three children had oral glucose tolerance tests and intravenous insulin tolerance tests performed Diabetic glucose tolerance was noted in 3 children None of them showed symptoms of diabetes mellitus neither was there any family history of diabetes One of these patients had experienced hypoglycaemia after the newborn period and responded with hyperinsulinism during the glucose tolerance test The other hypoglycaemic patient showed an exaggerated insulin release in response to tolbutamide Deficient serum cortisol response to insulin induced hypoglycaemia was demonstrated in 7 patients and 6 of these had concomitant minimal growth hormone response One of these patients also had a diabetic glucose tolerance None were of short stature It is probable that a disturbance in the hypothalamic pituitary adrenal axis may contribute to an impaired carbohydrate metabolism in some patients with neonatal hypoglycaemia

KEY WORDS Neonatal hypoglycaemia Idiopathic hypoglycaemia adrenal insufficiency diabetes mellitus

Hypoglycaemia is often encountered in low birth weight infants during the neonatal period and most frequently in those small for gestational age Later in infancy hypoglycaemia is a relatively rare condition Some hypoglycaemic syndromes may have their onset during the first few days after birth such as leucine sensitive hypoglycaemia hyperplasia of the islands of Langerhans and certain types of inborn errors of metabolism

The present investigation was carried out in order to disclose possible evidence of any persistent abnormalities in the carbohydrate metabolism in patients who had been hypoglycaemic during the first days of life

MATERIAL AND METHODS

During the three year period 1967–69 hypoglycaemia was registered in 67 newborn infants at the Department of Paediatrics University of Bergen The patients were classified in three groups according to clinical assessment Thirteen patients (group A) had *asymptomatic* hypoglycaemia Eleven cases (group B) were classified as *symptomatic transient* hypoglycaemia defined as symptomatic hypoglycaemia responding to glucose infusions and with no other apparent neonatal disorder Hypoglycaemia encountered simultaneously with neonatal complications such as asphyxia brain injury respiratory distress and with unsatisfactory response to glucose infusions was classified as *secondary* hypoglycaemia (group C 43 cases)

Further clinical details regarding the neonatal period of these patients have been published elsewhere (6)

Nineteen patients died during the neonatal period and one died later in infancy Nine cases were lost at follow up and one patient was excluded because of Down s syndrome Thus 37 patients were seen at follow up Mean age was 3 $^1/_2$ years range 2 6/12 – 4 9/12 years

Fasting blood glucose level was estimated in all patients Tolerance tests were carried out in every patient in group A (7) and in all but one in group B (8) From group C 8 patients treated during the year 1967 were selected for the study Oral glucose tolerance test with 1 75 g glucose per kg body weight was carried out on the day after admission Two days later an insulin tolerance test was undertaken

Table 1 *Oral glucose tolerance test at follow up in neonatal hypoglycaemia (means ±1 S D)*

Category of hypoglycaemia	No of subjects	Blood glucose mg/100 ml			Plasma insulin ng/ml				Growth hormone ng/ml		FFA μEq/l		
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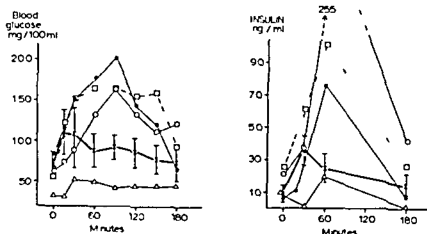


Fig 1 Blood glucose and plasma insulin values in 4 patients with pathological glucose tolerance One patient belonging to group A (●—●) 2 patients from group B R D (Δ—Δ) and I L (O—O) and one patient from group C (□ -□) Heavy line mean ±1 S D in controls

tient (R D) had a flat glucose curve starting at a hypoglycaemic level of 33 mg/100 ml

Blood glucose growth hormone cortisol and FFA responses to the intravenous insulin tolerance test are presented in Table 2 The growth hormone response showed wide individual variations mean increment was 4 7 7 2 4 2 ng/ml in groups A B and C respectively and 3 0 ng/ml in controls The differences were not statistically significant In group A children the mean serum cortisol level at 60 minutes remained at about the same level as in the fasting state while the patients in the other groups including the controls showed a considerable increase The mean cortisol increment in group A was 0 8 μg/100 ml which is significantly lower than the mean values in group B (5 1) in group C (9 3) and in controls (8 9) $p<0\ 01$ The difference between groups B and C was probably significant ($p<0\ 05$) but there were no significant differences between these groups and the controls When the individual responses were analysed it appeared that 7 patients did not show an increase in serum cortisol in the face of marked fall in the blood glucose level Blood glucose growth hormone and serum cortisol values from these patients are shown in Fig 2 The serum level of FFA showed no consistent pattern A moderate increase was noted in 4 patients whereas the remaining 3 showed a moderate fall

None of the 23 patients investigated developed symptomatic hypoglycaemia during 24 hours fasting Mean blood glucose levels at the end of the test were 54 mg/100 ml in group A 64 mg/100 ml in group B and 97 mg/100 ml in

Table 2 *Intravenous insulin tolerance test at follow up in neonatal hypoglycaemia (means ±1 S D)*

Category of hypoglycaemia	No of subjects	Blood glucose mg/100 ml 0[a]	min level	120	Growth hormone ng/ml 0	30	Serum cortisol μg/100 ml 0	60	FFA μEq/l 0	60	120
Asymptomatic	7	77 ± 9	46 ±11	71 ±12	3 9 ±3 9	8 6 ± 7 5	19 1 ±4 2	20 0 ±2 9	345 ±100	270 ±57	330 ±136
Symptomatic transient	8	76 ±12	36 ± 5	71 ± 9	1 9 ±2 0	9 1 ±11 5	14 4 ±5 3	19 5 ±9 4	335 ±173	203 ±84	3 8 ±140
Secondary	8	81 ±13	48 ±11	72 ±13	0 9 ±1 5	5 1 ± 5 2	17 8 ±3 8	27 5 ±9 8	424 ±196	331 ±25	340 ± [illegible]
Controls	5	84 ± 7	47 ±15	76 ± 9	6 1 ±7 7	9 1 ± 9 1	14 9 ±2 8	23 9 ±4 3	310 ±107	171 ± 7	309 ± 95

[a] All time points are given in minutes

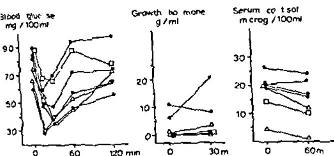


Fig 2 Blood glucose serum growth hormone and cortisol values during in travenous insulin tolerance test in 7 patients with impaired cortisol response Four pa tients from group A (●—●) 2 patients from group B (Δ—Δ) and one patient from group C (□—□)

group C Only one patient showed a hypo glycaemic value (I L) Overnight fasting blood glucose values were normal in the re maining 14 cases who were not included in the endocrinological investigations

The findings on intravenous tolbutamide tests and oral leucine tolerance tests in patients R D and I L are presented in Tables 3 and 4

Table 3 *Intravenous tolbutamide tolerance test in two patients at follow up in neonatal hypoglycaemia*

Time (min)	Patient R D	Patient I L
Bl od glucose mg/100 ml		
0	10?	74
5	97	66
10	93	61
?0	80	54
30	58	5
60	6?	57
90	70	50
1 0	35	5?
G o th hormon ng/ml		
0	6	10 5
30	–	19 8
60	5 5	15 5
1 0	? 6	1 4
Plasma insul n ng/ml		
0	40	40
30	>3 0	40
60	4? 5	0
1 0	3 5	5
FFA µEq/l		
0	593	370
30	00	141
60	331	81
1?0	4 7	5 8

Hyperinsulinism was evident in patient R D during the tolbutamide test with a peak plasma insulin value of 320 ng/ml at 30 minutes return ing to the fasting level at 60 minutes His blood glucose level was 35 mg/100 ml at 120 minutes During the leucine tolerance tests no signifi cant fall in blood glucose was noted (3) no hypoglycaemic symptoms were observed and the serum insulin remained unchanged

DISCUSSION

The incidence of hypoglycaemic attacks after the newborn period was 3 out of 37 patients investigated in this material A somewhat higher frequency (5 out of 24) has been reported by Eeg Olofsson and co workers (5) in a follow up study of neonatal symptomatic hypoglycaemia while Creery (4) found 2 cases among 17 survivors from neonatal hypo glycaemia The present series include both asymptomatic and secondary neonatal hypo glycaemia and in the latter group a lower frequency of later attacks would be expected When only symptomatic transient neonatal hypoglycaemia is considered the incidence of later hypoglycaemic episodes in the present material is 2 out of 9 patients which is in accordance with the figures of Eeg Olofsson et al (5)

In the present material a diabetic response to an oral glucose load was detected in 3 out of 23 children studied These infants belonged to

Table 4 *Oral leucine tolerance test in two patients with hypoglycaemic attacks after the newborn period*

Time (min)	Patient R D	Patient I L
Blood glucose mg/100 ml		
0	99	85
15	60	90
30	55	83
45	52	80
60	51	78
90	60	80
120	67	61
Growth hormone ng/ml		
0	3.3	4.0
30	4.7	2.7
60	3.3	2.2
120	5.3	5.1
Plasma insulin ng/ml		
0	45	55
30	70	40
60	65	25
120	65	27.5
FFA µEq/l		
0	176	244
30	108	185
60	89	200
120	79	193

different patient categories and the perinatal history showed no consistent pattern One of these patients had obviously had hypoglycaemic attacks beyond the newborn period An exaggerated insulin release was noted during the glucose tolerance test whereas the responses to tolbutamide and leucine were normal The other two patients had not had hypoglycaemic episodes after the newborn period However their insulin secretion during the test exceeded that of the controls and the peak value was reached at 60 min compared with 30 min in controls None of these patients have so far shown glucosuria or any other symptoms of diabetes mellitus and they have no family history of diabetes

An association between idiopathic hypoglycaemia and subsequent development of diabetes mellitus has been reported by several authors (10 11 16 17 18) In a series of 10 children from 9 families with idiopathic hypoglycaemia reported by Rosenbloom & Sherman (16) 5 out of 7 patients studied showed an abnormal hyperglycaemic response to glucose Furthermore, 8 of 9 probands had a family history of diabetes mellitus which is a frequency comparable to that found in individuals with diabetes mellitus Glucose tolerance tests in siblings and parents provided further evidence for diabetes mellitus in seven of the nine families studied Thus spontaneous hypoglycaemia in infancy may be an early manifestation of an inherited metabolic abnormality associated with diabetes mellitus

The interval between hypoglycaemic attacks and development of diabetes mellitus may be several years The reason diabetes mellitus is preceded by hypoglycaemia in these patients is not clear Plasma insulin levels during tolerance tests have seldom been determined in the reported cases Seltzer et al (17) postulated that the early defect in diabetes mellitus is a sluggish initial release of insulin by the islet cells which however ultimately respond to prolonged hyperglycaemia with excessive production of insulin This in turn leads to hypoglycaemia This hypothesis accounts satisfactorily for hypoglycaemic episodes occurring in the postprandial period but offers no explanation for hypoglycaemic episodes occurring in the fasting state An alternative hypothesis proposed by Lloyd (11) suggests the possibility of a temporary imbalance between the amounts of insulin and antagonists in such patients

Impaired cortisol response to insulin induced hypoglycaemia was detected in seven patients and one of these also showed diabetic glucose tolerance During recent years there have been several reports of adrenocortical unresponsiveness to insulin induced hypoglycaemia in children with idiopathic spontaneous hypoglycaemia (8 12 15 19) In a series of 14 patients studied by Rochiccioli and co authors (15) there was a history of neonatal complications in 8 cases including 3 infants with documented hypoglycaemia

However we are unaware of other studies which have uncovered a similar adrenocortical unresponsiveness during follow up of apparently normal children with a history of neonatal hypoglycaemia and it is noteworthy that 4 out of 7 cases in our series had asymptomatic neonatal hypoglycaemia In children with spontaneous idiopathic hypoglycaemia the defective response of the adrenal cortex to insulin induced hypoglycaemia has been encountered as an isolated phenomenon (12 15) or in combination with an impaired release of epinephrine from the adrenal medulla (8 15 19) The latter finding has given rise to speculations that the hypothalamus is the site of the primary disorder in these patients resulting in a dysregulation of both the adrenal medulla and cortex (8 19) Such an explanation is substantiated by the finding of deficient ACTH release in one patient (8) However in 3 patients studied by Martin & Martin there was an adequate catecholamine release in response to insulin induced hypoglycaemia concomitant with no response in plasma cortisol or plasma ACTH (12)

In the majority of patients with spontaneous hypoglycaemia there has been a normal response of growth hormone to insulin induced hypoglycaemia It will be noted that the growth hormone response was minimal in spite of an adequate hypoglycaemic stimulus in all but one of our patients with deficient adrenocortical response None of these patients were of short stature This finding might lend some support to the hypothesis of Nakagawa & co workers (14) who propose that ACTH and growth hormone released in response to insulin induced hypoglycaemia result from the stimulation of a common hypothalamic center In that event the deficient cortisol response in our patients could be explained as a probable disturbance in the hypothalamic regulatory mechanisms rather than a primary adrenal defect Conceivably such a disturbance could have been present in the neonatal period and contributed to the hypoglycaemia

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REFERENCES

1 Catt K & Tregear G W Solid phase radioimmunoassay in antibodycoated tubes *Science* 158 1570 1967
2 Duncombe W G The colorimetric microdetermination of non-esterified fatty acids in plasma *Clin Chim Acta* 9 122 1964
3 Cornblath M & Schwartz R Hypoglycaemic syndromes in infancy *In* M Cornblath & R Schwartz (eds) *Disorders of carbohydrate metabolism in infancy* Saunders Philadelphia and London 1967 p 193
4 Creery R D G Hypoglycaemia in the newborn Diagnosis treatment and prognosis *Develop Med Child Neurol* 8 746 1966
5 Eeg Olofsson O Gentz J Jodal U Nilsson L R & Zetterstrom R Neonatal symptomatic hypoglycaemia Some results from a follow up study *Acta Paediatr Scand* Suppl *177* 85 1967
6 Fluge G Clinical aspects of neonatal hypoglycaemia *Acta Paediatr Scand* *63* 826 1974
7 Hultman E Rapid specific method for determination of aldosacchandes in body fluids *Nature 183* 108 1959
8 Hung W & Migeon C J Hypoglycaemia in a two-year old boy with adrenocorticotropic hormone deficiency and adrenal medullary unresponsiveness to insulin induced hypoglycaemia *J Clin Endocrinol Metab 28* 146 1968
9 Hyvarinen A & Nikkila E A Specific determination of blood glucose with o toluidine *Clin Chim Acta* 7 140 1962
10 Lister J The clinical spectrum of juvenile diabetes *Lancet I* 386 1966
11 Lloyd J K Diabetes mellitus presenting as spontaneous hypoglycaemia in childhood *Proc R Soc Med 57* 1061 1964
12 Martin M M & Martin A L A Idiopathic hypoglycaemia—a defect in hypothalamic ACTH releasing factor secretion *Pediatr Res* 5 396 1971
13 Murphy B E P Clinical evaluation of urinary cortisol determinations by competitive protein binding radioassay *J Clin Endocrinol Metab 28* 343 1968
14 Nakagawa K Horiuchi Y & Mashimo K Further studies on the relation between growth hormone and corticotrophin secretion in insulin induced hypoglycaemia *J Clin Endocrinol Metab 32* 188 1971
15 Rochiccioli P Ribot C Dutau G & Dalous A Hypoglycemie par deficit cortico et/ou medullo-surrenalien *Arch Fr Pediatr 30* 977 1973
16 Rosenbloom A L & Sherman L The natural

history of idiopathic hypoglycaemia of infancy and its relation to diabetes mellitus N Engl J Med 274 815 1966
17 Seltzer H S Fajans S S & Conn J W Spontaneous hypoglycaemia as an early manifestation of diabetes mellitus *Diabetes 5* 437 1956
18 Sperling M & Drash A Evolution of diabetes mellitus from hypoglycaemia *Am J Dis Child 121* 5 1971
19 Tietze H U Zurbrugg R P Zuppinger K A Joss E E & Kaser H Occurrence of impaired cortisol regulation in children with hypoglycaemia associated with adrenal medullary hyporesponsiveness *J Clin Endocrinol Metab 34* 948 1972
20 Wide L Axèn R & Porath J Radioimmunosorbent assay for proteins Chemical couplings of antibodies to insoluble Dextran *Immunochemistry 4* 381 1967

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THE EFFECT OF VARIOUS THERAPEUTIC TRIALS ON THE PORPHYRIN EXCRETION IN A CASE OF CONGENITAL ERYTHROPOIETIC PORPHYRIA

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KEY WORDS Erythropoietic porphyria photosensitivity hemolytic anemia splenectomia vitamin E β-carotene erythropoiesis inhibiting factor (EIF) erythropoietic chalone

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In the present paper the effects of different therapeutic trials (packed red cell transfusion splenectomy vitamin E β-carotene)

Table 1 *Amounts and types of porphyrins excreted daily in the urine in pretreatment periods and after response to treatment*

Type of treatment	Vitamin E						Red cell transfusion					
Days after start	0		6		14		0		4		10	
Porphyrin	μg	% III	μg	% III	μg	% III	μg	% III	μg	% III	μg	% III
copro	870	20	650	20	400	20	1 200	20	370	20	700	20
isocopro	400		300		200		600		150		100	
penta	780	50	450	40	350	40	700	40	150	40	150	40
hexa	870	95	500	95	350	95	850	95	180	95	190	95
hepta	4 330	100	2 800	100	2 500	100	4 450	100	1 000	100	840	100
uro	4 330	30	2 900	30	2 500	30	4 600	30	1 000	30	860	30
Total	11 570	50	7 600	50	6 300	50	12 400	50	3 800	50	2 300	50

in this patient on the urinary porphyrin excretion as compared to the photosensitivity of the skin and the tendency to develop hemolytic anemia when exposed to unfiltered daylight will be presented Evidence supporting our previous suggestion that the biochemical defect in porphyrin metabolism is located to a production line of shortlived erythrocytes will also be presented

MATERIALS AND METHODS

The patient a boy born on July 8 1969 has been under continuous study since August 1970

The techniques used for the extraction separation and quantitation of porphyrins have been described in previous papers (1 5) Otherwise routine hematological methods have been used

The photosensitivity of the skin was until July 1973 evaluated by the tendency to develop skin eruptions on exposure to unfiltered daylight avoiding direct sunlight Since July 1973 measurements have been made at the Department of Dermatology University Hospital Oslo with filtered light from an Osram High Pressure Xenon Arc Lamp (XBO 150 W) delivering 870 erg/sec/cm² at 410 nm

A Zetopane fluorescence microscope with Binolux (Reichert Vienna) was used for dark field fluorescence microscopy in blue light (12)

Vitamin E was obtained from Farmaceutisk Industri a/s Oslo and dispensed as pellets or in suspension

β carotene capsules with canthaxanthine were supplied by Hoffman LaRoche

RESULTS

Light shielding We have previously shown (2) that effective shielding against light of wavelengths below 510 nm not only removed the troublesome skin lesions but also brought about an almost complete compensation of the anemia and disappearance of the splenomegaly During light shielding the

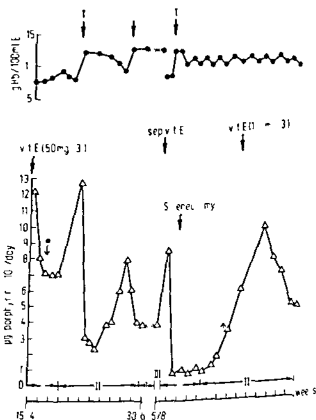


Fig 1 The effect of vit E red cell transfusions (T) and splenectomy on urinary prophyrin excretion (Δ) and hemoglobin level (●) under varying degrees of light shielding (I=effective II=ineffective but no sun III=travel with solar exposure) ●↑=skin rash appeared ●↓=skin rash disappeared

splenectomy					
		30		150	
μg	% III	μg	% III	μg	% III
170	70	800	20	1 300	70
50		*400*		*700*	
60	50	550	40	750	50
70	95	650	95	800	95
450	100	2 500	100	5 000	100
450	30	2 500	30	*5 050*	*30*
700	50	7 400	50	14 600	50

porphyrinogen/porphyrin ratio in the uterine increased markedly but the total amount of porphyrin excreted remained unchanged

Red cell transfusion The effect of red cell transfusions on porphyrin excretion is shown in Fig 1 We see that on three different occasions transfusions with *normalization of the* hemoglobin level were followed in 4–5 days by a dramatic fall in porphyrin excretion In connection with splenectomy two transfusions were given and the fall in porphyrin excretion was particularly striking and prolonged ACD blood from the blood bank (stored 1–3 days in one instance Aug 1972 21 days) was used and the amounts given were 250 ml whole blood *plus 100 ml* packed cells in May 1972 100 *ml packed* cells in June 1972 and 250 ml packed cells plus 120 ml whole blood in August 1972

Splenectomy Before splenectomy the porphyrin excretion was brought down by transfusions *and* the low porphyrin excretion in the *immediate* postoperative period can be explained by the transfusions (Fig 1) After a few weeks the porphyrin excretion increased to the previous levels The clinical picture photosensitivity *and hemoglobin* level were also uninfluenced by splenectomy

Vitamin E In April 1972 in a period when the patient was well protected against light treatment with vitamin E 50 mg three times daily perorally was started The hemoglobin level and the daily excretion of porphyrins were determined one to three times weekly (Fig 1) In 8 days the porphyrin excretion dropped to approximately 55–60% of the initial value and stabilized Exposure to moderate doses of unfiltered daylight lead to a rapid increase in porphyrin excretion A second therapeutic trial with vitamin E (100 mg three times daily) from Sept 1972 to May 1973 (Fig 1) resulted in a moderate but significant drop in porphyrin excretion However there was no obvious improvement of the photosensitivity and the hemolytic anemia

β-carotene In May 1973 treatment with β-carotene capsules with canthaxanthine *25 mg daily was begun (Fig 2) The beneficial effect of β carotene on skin photosensitivity has been reported in a previous paper* (14) During this *treatment it* has been impossible to *maintain meticulous light* shielding since the boy knows that he is

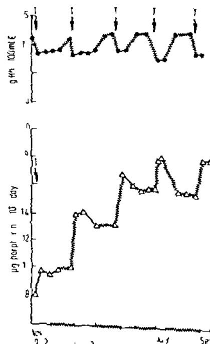


Fig 2 The effect of β-carotene (*I*) on urinary porphyrin excretion (Δ) and hemoglobin level (●) *T*=travel with solar exposure ——— hospitalized ~~~~~ at home

able to tolerate more light than before The porphyrin excretion has remained essentially unchanged in relation to his body weight and has been higher than during vitamin E therapy His hemoglobin level at home has been somewhat higher than earlier but each time he has travelled to the hospital and thereby been exposed to sunshine while travelling a significant fall has occurred and at the same time the colour of the urine has changed from yellowish to dark brownish red

It will be seen from Table 1 that the various forms of treatment had no significant effects on the relative amounts of different porphyrins (uro hepta hexa penta iso copro and coproporphyrin) excreted in the urine The percentage of porphyrins belonging to the isomer III series also remained unchanged throughout

Fluorescence microscopy of the bone marrow showed a large number of fluoroblasts with the fluorescence primarily located in the nuclei The degree of fluorescence increased with increasing maturity A great number of mononuclear phagocytes were found to contain material with stable fluorescence This material was found in the cytoplasm only Stable fluorescence was also found in extracellular material in these smears When a drop of physiological saline was added to the slides and the slides covered with a coverglass a couple of minutes prior to exposure it was found that almost all the erythroid cells present in the marrow showed a rapidly fading red fluorescence in their cytoplasm

A considerable number of fluorocytes and varying number of fluoroblasts were found in the peripheral blood

DISCUSSION

Rimington & With (11) based on studies of material from our patient suggested that he may represent an erythrohepatic type of porphyria and that the liver contributes substantially to the overall porphyrin excretion However, the rapid and marked depression of porphyrin excretion achieved by red cell transfusions seems to exclude this possibility and supports the view that the liver as also seems to be the case in classical CEP (10) plays no or at most a minor role in the abnormal porphyrin metabolism of our patient The rapidity of the fall in porphyrin excretion after transfusion indicates that the bulk of the excreted porphyrins originates from a population of shortlived red cells

It could be objected that the liver although it has relatively little effect on the total amount of porphyrins excreted, might influence the composition of the excreted porphyrins However the findings presented in Table 1 that red cell transfusions even though they produce a strong depression of the total amount of urinary porphyrin have no influence on the relative amounts of the different types of porphyrins excreted are strong evidence that the abnormal porphyrins are erythropoietic in origin

The reported findings on fluorescence microscopy of blood and bone marrow are also in accord with this view It could be mentioned that the great number of fluorescing macrophages demonstrated in bone marrow is in agreement with observations in classical CEP (4) These observations support the assumption that pathological red cells are to a great extent destroyed in the bone marrow They also explain why the amount of so-called early labelled bile pigment is increased in CEP even in periods in which anemia is absent or moderate (8) This bile pigment has been found to originate from erythroid cells in CEP (9)

When the patient s hemoglobin level is brought up to about 12 g/100 ml by transfusion the urinary porphyrin excretion falls to one tenth of the pretransfusion level in 4–5 days However a similar increase in hemoglobin as a result of improved survival of his own red cells in periods of meticulous light shielding has no effect on the total

amount of porphyrins excreted (2) This discrepancy is quite remarkable The inhibitory effect of red cell transfusion on porphyrin formation is at least in part due to a depression of erythropoietin production with subsequent reduction of the patient s erythropoietic activity It is reasonable to assume that erythropoietin production is reduced also when the hemoglobin level rises as a result of effective light shielding A possible explanation of the above mentioned discrepancy could be that the older more mature red cells in transfused blood exert an inhibitory effect on erythropoiesis which is *not* mediated through the effect on erythropoietin formation and which is not exerted by the patient s much younger pathologic red cells It is tempting to speculate that the erythropoiesis inhibiting factor (EIF) studied by Lindemann (6) and by others is as suggested earlier (13) an erythrocytic chalone produced by the red cells and that this factor is formed mainly in the oldest most mature red cells A mechanism of this type would explain the overshooting seen in recovery from anemias where increased production of red cells continues for days after an adequate hemoglobin level has been reached and also the phenomenon of compensated hemolytic anemias It is of interest in this connection that no EIF could be demonstrated in the urine of our patient (7) It could be argued that hematin present in the infused blood could possibly have a repressive effect on porphyrin synthesis but we find it very unlikely that this can explain the marked drop in porphyrin excretion observed since the amounts of hematin infused must have been modest

Murty et al (9) reported vitamin E to depress urinary porphyrin excretion and reduce the photosensitivity in *porphyria cutanea tarda symptomatica* but others have been unable to confirm this (15) Since the porphyrin excretion pattern of our patient has similarities to that observed in *porphyria cutanea tarda* it was decided to try massive doses of vitamin E A moderate reduction in porphyrin excretion was achieved However vitamin E in the doses given had no or negligible effect on skin photosensitivity and no effect on the tendency to develop hemolytic anemia

β carotene medication has been beneficial in reducing photosensitivity in our patient as previously reported (14) but has no effect on the total amount of porphyrins excreted in the urine (Fig 2) and no or only slight effect on the tendency to develop hemolytic anemia It therefore seems unreasonable to assume that β-carotene by accumulation in the skin should act as a simple filter removing the destructive wavelengths since this should give a similar protection of the circulating red cells It seems more likely that β-carotene has a local quenching effect on photocatalytically formed radicals thereby protecting cell structures and enzymes The inability of β-carotene to prevent the tendency to develop hemolytic anemia might be due to too low concentration of the drug in or on the erythrocytes to protect them against photochemical injury

Splenectomy which may be helpful in certain cases of classical CEP had no demonstrable effect in our patient

REFERENCES

1 Eriksen L & Eriksen N Porphyrin distribution and porphyrin excretion in human congenital porphyria *Scand J Clin Lab Invest* in press
2 Eriksen L Hofstad F & Seip M Congenital erythropoietic porphyria The effect of light shielding *Acta Paediatr Scand* 62 385 1973
3 Eriksen L & Seip M Congenital erythropoietic porphyria A family study *Clin Genet* 4 166 1973
4 Heilmeyer L Clotten R Kerp L Merker H Parra C A & Wetzel H P Porphyria erythropoietica congenita Gunther Bericht uber zwei Familien mit Erfassung Merkmalstrager *Dtsch Med Wochenschr* 88 2449 1963
5 Hofstad F Seip M & Eriksen L Congenital erythropoietic porphyria with a hitherto undescribed porphyrin pattern *Acta Paediatr Scand* 62 380 1973
6 Lindemann R Erythropoiesis inhibiting factor (EIF) Fractionation and demonstration of urinary EIF *Br J Haematol* 21 632 1971

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STUDIES ON PHOTOTHERAPY IN NEWBORN INFANTS

Influence on Protein Binding of Bilirubin and Salicylate and on Activity of Acetylsalicylic Acid Esterase

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ABSTRACT Windorfer A Faxelius G and Boreus L O (Departments of Clinical Pharmacology and Paediatrics Karolinska Hospital Stockholm Sweden) Studies on phototherapy in newborn infants Influence on protein binding of bilirubin and salicylate and on activity of acetylsalicylic acid esterase Acta Paediatr Scand 64 293 1975.—Phototherapy of newborn infants with hyperbilirubinemia was shown to result in an increase in hematocrit values and in the activity of the erythrocyte enzyme acetyl salicylic acid esterase The elevation of the enzyme activity also could be produced in light treated rabbits and *in vitro* after illumination of blood from adult volunteers The binding of bilirubin to serum albumin and of salicylate to plasma proteins did not alter nor did the concentrations of albumin or total proteins in plasma It is concluded that light does not increase the unbound fraction of bilirubin in blood

KEY WORDS Newborn infants hyperbilirubinemia phototherapy protein binding salicylates

It is now well established that light (400–450 nm) can depress the serum bilirubin levels in newborns with hyperbilirubinemia (1 3 4 5) The mechanism of the effect is still unknown The photo-oxidation of the unconjugated bilirubin yields polar metabolites which according to Ostrow (9) have no neurotoxicity in laboratory animals However Ostrow & Branham (11) demonstrated that the metabolites formed *in vitro* during light treatment are not identical with those formed *in vivo*

In order to evaluate light treatment clinically both short term and long term studies on its mechanism and effects are badly needed To date very few such studies have been performed Odell et al (7) reported a decrease in albumin bilirubin binding after light treatment Since his experiments were made *in vitro* it was considered to be of interest to see whether the binding is influenced by light treatment under clinical conditions This has been done in the present paper which includes data on binding both of bilirubin and salicylate before and after phototherapy in newborns with hyperbilirubinemia The effect of light on the erythrocyte bound enzyme acetylsalicylic acid esterase has also been studied For comparison parallel experiments were also done on light treated rabbits as well as on light treated blood and plasma *in vitro*

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MATERIAL AND METHODS

(A) Studies on newborns

Blood was obtained from 20 newborns of whom 15 were subjected to phototherapy for 2–4 days because of hyperbilirubinemia of unclear etiology Birth weights ges

7 — Personal communication
8 Marver H S & Schmid R The porphyrias *In* J B Stanbury W B Wyngaarden & D S Frederickson (eds) *The metabolic basis of inherited disease* McGraw Hill New York 1972 p 1087
9 Murty H S Pinelli A Nair P P & Mendelhoff A I Porphyria cutanea tarda therapeutic response to vitamin E *Clin Res 17* 474 1969
10 Nicholson D C Cowger M L Kalivas J Thompson R P & Gray C H Isotopic studies of the erythropoietic and hepatic components of congenital porphyria and erythropoietic protoporphyria *Clin Sci 44* 135 1973
11 Rimington C & With T Porphyrin studies in congenital erythropoietic porphyria *Dan Med Bull 20* 5 1973
12 Runge W & Watson C J The effect of bleeding on the proportion of red fluorescing forms among total normoblasts of bovine porphyric bone marrow *Blood 33* 119 1969
13 Seip M Erythropoietic inhibiting factor (EIF)— an erythrocytic chalone? *Ann Clin Res 3* 3 1971
14 Seip M Thune P O & Eriksen L Treatmen of photosensitivity in congenital erythropoietic por phyria (CEP) with beta-carotene *Acta Derm Venero* (Stockh) *54* 239 1974
15 Watson C J Bossenmaier I & Cardinal R Lack of significant effect of vitamin E on porphyri metabolism *Arch Intern Med 131* 698 1973

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Table 2 *Results of blood analyses before and after phototherapy in newborn infants*

Before and after denote blood samples taken 2–4 days apart
I=albumin bilirubin molar ratio 1 0 5 II=albumin bilirubin molar ratio 1 1

Patient	Protein binding of salicylate %		Total serum bilirubin (mg/100 ml)		Unbound bilirubin (% of total* bilirubin)				Serum albumin (g/100 ml)	
					Before		After			
	Before	After	Before	After	I	II	I	II	Before	After
(A) *With phototherapy*										
1	36	34	18 2	14 5	1 2	1 6	1 4	1 6	3 45	3 3
2	40	45	13 8	12 4	1 4	2 0	1 4	1 8	3 9	4 0
3	32	28	13 4	11.5	1 1	1.5	1 3	1 5	3 5	3 3
4	33	35	19 5	11 2	1 35	1 8	0 9	1 7	3 5	3 7
5	26	29	20 0	11 0	1 1	1 5	0 8	1 1	3 3	3 4
6	41	43	16 6	13 5	1 2	1 7	1 1	1 8	3 1	2 9
7	40	43	19 3	13 2	0 85	1 2	0 9	1 2	2 9	3 0
8	43	44	18 0	14 2	1 05	1 5	1 15	1 5	3 5	3 5
9	38	36	19 2	13 6	1 0	1 35	1 0	1 4	3 9	3 8
10	42	40	18 5	14 0	0 75	1 2	0 9	1 3	4 0	3 9
11	42	45	20 2	14 3	1 1	1 7	1 0	1 5	3 3	3 5
12	18	20	15 1	12 7	1 6	2 1	1 5	2 1	2 9	2 7
13	27	26	19 2	13 6	1 3	2 0	1 4	2 0	3 1	3 0
14	32	34	19 5	15 1	1 1	1 7	1 3	1 8	2 9	2 9
15	37	25	18 1	12 5	1 5	2 2	1 7	2 5	3.5	2 8
(B) *Without phototherapy*										
1	38	39			1 0	1 4	1 1	1 5	3 4	3 45
2	45	41			1 2	1 6	1 2	1 5	3 1	3 0
3	35	37	12 5		0 9	1 3	0 8	0 3	3 8	4 0
4	32	35	13		1 2	1 7	1 4	1 8	3 2	2 9
5	40	37			0 8	1 3	0 8	1 4	2 9	3 0

Unbound is the bilirubin eluted from the Sephadex column
* Total bilirubin denotes the sum of bilirubin originally present in the sample and the added amount

varying albumin and bilirubin concentrations The unbound bilirubin is expressed as a percentage of the final total bilirubin concentration (bilirubin originally present in the sample+added bilirubin) A detailed description of the method has been published earlier (16 17)

(4) Determination of the protein–salicylate binding was done after addition of 160 μg/ml sodium salicylate to 0 5 ml serum Determination of unbound and bound salicylate was performed according to Potter & Guy (13) by separation on Sephadex G 25 *(5)* Determination

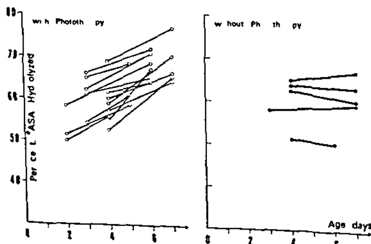


Fig 2 Acetylsalicylic acid esterase activity expressed as percentage acetylsalicylic acid hydrolysed before and after phototherapy (left) An increase in activity is not seen in the controls (right)

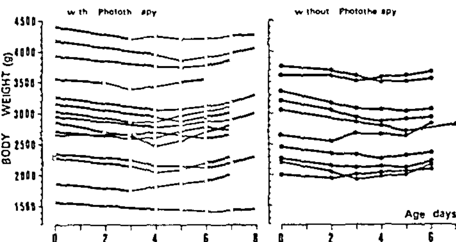


Fig 1 Changes in body weight in light treated newborn infants (left) and in controls (right) The period of phototherapy is denoted by a thinner line

tational ages and presenting diagnoses are given in Table I No infant with hyperbilirubinemia from hemolytic disease were included All the infants were admitted to the neonatal ward Five newborns from the same ward were used as control patients In the comparison of birth weights the control group could be increased to 10 newborns The control babies were admitted because of cyanotic spells diabetic fetopathia or neonatal icterus not needing treatment In connection with blood sampling for diagnostic purposes 2 ml heparinized blood and 2–3 ml blood without anticoagulant was taken before and after light treatment The same sampling was done in the control infants Blood which showed visual hemolysis was not used

Light treatment was performed at intervals (6 hours light 2 hours without light) This treatment was started when the total bilirubin on the second day of life exceeded 10 mg/100 ml or on the third day exceeded 14 mg/100 ml (6) An Airshield phototherapy lamp was used with 8 daylight 20-watt lamps which gave 4 000 lux (370 footcandles)

(B) Studies on rabbits

Male albino rabbits were shaved on the back and lateral aspects of the body Light treatment was instituted on the following day and lasted 3–4 days without intervals by means of 2 white 20-watt daylight lamps (2 000 lux = 185 footcandles) at a distance of 50 cm above the rabbit No cover over the eyes was used Before and after the light treatment 1 ml venous blood was taken Free water intake was allowed and measured

(C) Studies on blood and plasma in vitro

Heparinized blood was drawn from the cubital vein of 5 male adult volunteers Five ml blood or plasma was placed in Petri dishes with plastic covers and exposed to light for 20 hours Control samples were arranged in an identical way and during the same time period but placed in darkness All *in vitro* experiments were done at room temperature

Procedures

All samples were subjected to the following procedures (*1*) Determination of hematocrit (2) Determination of total protein and electrophoretogram Beckman Spinco photometer and microzone electrophoresis equipment were used (*3*) Determination of the albumin–bilirubin binding according to a method which has been described earlier (16 17) According to this method a constant amount of diazepam is added in order to achieve a bilirubin displacement at an albumin–bilirubin ratio of 1 0 5 and 1 1 This ratio was accomplished by adding a suitable amount of alkaline bilirubin solution to the sample The pH was then adjusted to exactly 7 4 Separation of unbound and bound bilirubin was performed with gel chromatography on Sephadex G 25 and the eluted unbound bilirubin determined by diazotation The result value for unbound bilirubin is higher than the real value in serum but gives good information on the degree of the albumin–bilirubin binding in a patient material with

Table I *Clinical data*

Patient	Birth weight	Gestational age	Presenting diagnosis (except hyperbilirubinemia)
(A) With phototherapy			
1	3 150	38	–
2	2 850	36	Purulent conjunctivitis
3	2 650	34	Aspiration
4	2 700	34	–
5	3 300	36	–
6	4 190	41	Maternal diabetes
7	3 [illegible]50	37	–
8	4 230	38	Cyanosis
9	4 400	37	–
10	3 860	36	–
11	3 040	38	–
12	1 560	32	Prematurity cyanosis
13	1 870	34	Prematurity
14	2 330	34	Prematurity
15	1 870	3[illegible]	Prematurity RDS
(B) Without phototherapy			
1	2 800	35	Cyanosis
2	3 770	37	Maternal diabetes
3	3 010	36	–
4	4 120	38	–
5	3 160	36	Atelectasis

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Before and after denote blood samples taken 2-4 days apart
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Patient	Protein binding of salicylate %		Total serum bilirubin (mg/100 ml)		Unbound bilirubin (% of total[a] bilirubin)				Serum albumin (g/100 ml)	
					Before		After			
	Before	After	Before	After	I	II	I	II	Before	After
(A) *With phototherapy*										
1	36	34	18 2	14 5	1 2	1 6	1 4	1 6	3 45	3 3
2	40	45	13 8	1? 4	1 4	2 0	1 4	1 8	3 9	4 0
3	3?	28	13 4	11 5	1 1	1 5	1 3	1 5	3 5	3 3
4	33	35	19 5	11 2	1 35	1 8	0 9	1 7	3 5	3 7
5	26	?9	?0 0	11 0	1 1	1 5	0 8	1 1	3 3	3 4
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3	35	37	1?.5		0 9	1 3	0 8	0 3	3 8	4 0
4	3?	35	13		1 2	1 7	1 4	1 8	3 2	?9
5	40	37			0 8	1 3	0 8	1 4	2 9	3 0

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(4) Determination of the protein-salicylate binding was *done after addition of 160 μg/ml* sodium salicylate to 0 5 ml serum *Determination* of unbound and bound salicylate was performed *according to Potter & Guy* (13) by separation on Sephadex G 25 (5) Determination

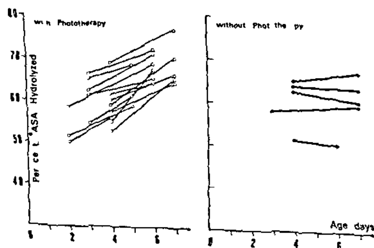


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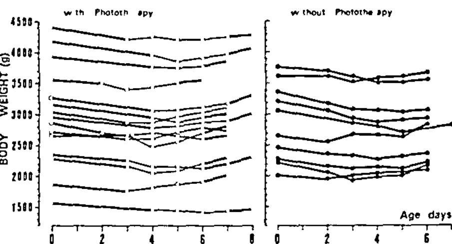


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	Before	After	Before	After	I	II	I	II	Before	After
(A) *With phototherapy*										
1	36	34	18 2	14 5	1 2	1 6	1 4	1 6	3 45	3 3
2	40	45	13 8	12 4	1 4	2 0	1 4	1 8	3 9	4 0
3	32	28	13 4	11 5	1 1	1 5	1 1	1 5	3 5	3.3
4	33	35	19 5	11 2	1 35	1 8	0 9	1 7	3 5	3 7
5	26	29	20 0	11 0	1 1	1 5	0 8	1 1	3 3	3 4
6	41	43	16 6	13 5	1 2	1 7	1 1	1 8	3 1	2 9
7	40	43	19 3	13 2	0 85	1 2	0 9	1 2	2 9	3 0
8	43	44	18 0	14 2	1 05	1 5	1 15	1 5	3 5	3 5
9	38	36	19 2	13 6	1 0	1 35	1 0	1 4	3 9	3 8
10	42	40	18 5	14 0	0 75	1 2	0 9	1 3	4 0	3 9
11	4?	45	20 2	14 3	1 1	1 7	1 0	1 5	3 3	3 5
1?	18	20	15 1	1? 7	1 6	2 1	1 5	2 1	2 9	2 7
13	27	26	19 2	13 6	1 3	2 0	1 4	2 0	3 1	3 0
14	32	34	19 5	15 1	1 1	1 7	1 3	1 8	2 9	2 9
15	37	25	18 1	12 5	1 5	2 2	1 7	2 5	3 5	2 8
(B) *Without phototherapy*										
1	38	39			1 0	1 4	1 1	1 5	3 4	3 45
2	45	41			1 2	1 6	1 2	1 5	3 1	3 0
3	35	37	12 5		0 9	1 3	0 8	0 3	3 8	4 0
4	3?	35	13		1 2	1 7	1 4	1 8	3 2	2 9
5	40	37			0 8	1 3	0 8	1 4	?9	3 0

ᵃ Unbound is the bilirubin eluted from the Sephadex column
ᵇ Total bilirubin denotes the sum of bilirubin originally present in the sample and the added amount

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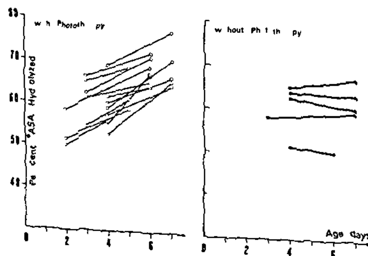


Fig 2 Acetylsalicylic acid esterase activity expressed as percentage acetylsalicylic acid hydrolysed before and after phototherapy (left) An increase in activity is not seen in the controls (right)

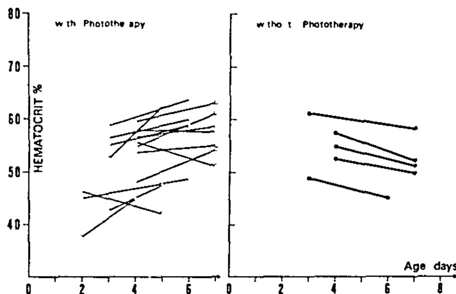


Fig 3 Changes in hematocrit values in light treated newborns (left) and in controls (right)

of the activity of the acetylsalicylic acid esterase Acetylsalicylic acid was added to 0 5 ml heparinized blood to achieve a final concentration of 200 μg/ml The mixture was incubated for 60 minutes and the salicylic acid formed determined after separation on Sephadex columns according to Potter & Guy (13) The fractions of protein–bound salicylate and unbound salicylate were determined on an Aminco Bowman photofluorimeter The activating wavelength was 300 nm and the emission wavelength 410 nm This means a slight deviation from the method described by Potter & Guy (13) The amount of hydrolysed acetylsalicylic acid per time unit was taken as a measure of enzyme activity

RESULTS

Newborn infants

The total serum bilirubin values decreased during light treatment Twelve out of the 15 newborns showed diarrhoea with loose and green stools This was not seen in any of the control children The frequency of defecation was 3–5 times a day in the light treated infants and 1–3 times a day in the patients without light treatment As seen in Fig 1 a similar decrease in body weight during the first week of life occurred in both groups No skin reaction from the light could be observed Some degree of somnolence in the light treated infants was regularly seen but it could not be determined whether this was due to the treatment or to the fact that the treated infants had higher bilirubin levels than the controls

Table 2 summarizes the results of the analyses in the infants Neither the albumin concentration nor the total protein concentration nor the total protein concentration changed during phototherapy and the electrophoresis pattern remained the same (In one child patient 15 there was a clear decrease in both total protein and albumin No explanation for this could be found)

The albumin–bilirubin binding values which were measured at two different bilirubin concentrations (molar ratio albumin-bilirubin 1 0 5 and 1 1) did not show any statistically significant difference before and after phototherapy

The binding of salicylate to plasma proteins was somewhat variable in all infants including the controls and no significant change during light treatment could be found Again patient number 15 was an exception (see Table 1)

A clear increase in acetylsalicylic acid esterase activity after light treatment was observed (Fig 2) This occurred in most of the light treated infants but in none of the control patients The increase was between 10 and 20%

The hematocrit values in the two groups of infants are shown in Fig 3 In 13 of the 15 light treated infants the hematocrit value increased and in two cases this reaction was especially great In contrast the hematocrit values decreased in the controls

The electrophoretic pattern and the

Table 3 *Results of blood analyses before and after phototherapy in rabbits*

Before and after denote blood samples taken 3 days apart
I=albumin bilirubin molar ratio 1 0 5 II=albumin bilirubin molar ratio 1 1

Animal	Hematocrit (%)		Protein binding of salicylate (%)		Esterase activity (%)		Unbound[a] bilirubin (% of total[b] bilirubin)				Serum albumin (g/100 ml)	
							Before		After			
	Before	After	Before	After	Before	After	I	II	I	II	Before	After
(A) *With phototherapy*												
Rabbit A	45	46	18	24	84	9[illegible]	1 3	2 15	1 5	2 0	3 7	3 5
Rabbit B	44	45	28	25	8[illegible]	87	1 1	2 15	1 6	2 2	4 1	3 9
Rabbit C	38	38	32	38	79	88	1.5	2 15	1 5	2 2	4 4	4 3
Rabbit D	43	48	28	24	81	88	1 45	2 1	1 7	2 3	4 3	3 7
(B) *Without phototherapy*												
Rabbit E	43	42	25	26	85	84	1 4	1 9	1 4	2 0	4 0	3 95
Rabbit F	46	44	31	29	78	78	1 5	2 05	1 5	2 0	4 2	4 3
Rabbit G	43	40	22	27	76	74	1 6	2 1	1 6	2 2	4 15	4 2
Rabbit H	38	39	2[illegible]	20	75	76	1 55	2 1	1 5	2 1	3 9	3 8

[a] Unbound is the bilirubin eluted from the Sephadex column
[b] Total bilirubin denotes the sum of bilirubin originally present in the sample and the added amount

centration of total protein in plasma was not influenced by the light treatment

Animal experiments (Table 3)

The increase in the hematocrit values seen in newborn infants could not be found in the rabbits The water intake was not significantly influenced by the light treatment

The starting values for protein binding of salicylates and for albumin binding of bilirubin were lower in the rabbits than in the infants Also in the animal experiments there was no change in protein binding of salicylate or bilirubin following light treatment As in the newborns the acetyl salicylic acid esterase activity increased in the rabbit following phototherapy No change in serum protein or albumin concentrations could be found

In vitro experiments

Hematocrit electrophoresis protein and albumin concentrations did not change after illumination of infant or rabbit blood However the esterase activity increased in both species after phototherapy also *in vitro* A slight statistically insignificant decrease in protein salicylate binding was seen The albumin–bilirubin binding did not change in any of the two bilirubin concentrations

DISCUSSION

The mechanism by which light exposition depresses the bilirubin levels in newborns is only partly known and it is still an open question if decreased total bilirubin levels really reflect a lower risk of kernicterus In fact the *in vitro* studies of Odell et al (7) which showed a decrease in the albumin–bilirubin binding after light treatment could be interpreted as allowing a facilitated penetration of bilirubin into the central nervous system Obviously measurements of albumin–bilirubin binding in newborns under phototherapy were necessary to clarify the situation Such a study was performed by Porto (12) who could not find any change in this binding However he used the HBABA method which according to Thaler & Schmid (14) and Bratlid (2) is not satisfactory

With the method used in the present paper there was no sign of decreased binding after phototherapy either in newborns or in rabbits Furthermore we could not reproduce

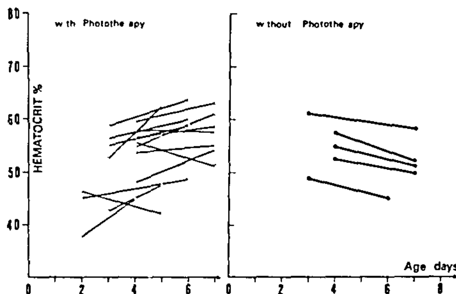


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Newborn infants

The total serum bilirubin values decreased during light treatment Twelve out of the 15 newborns showed diarrhoea with loose and green stools This was not seen in any of the control children The frequency of defecation was 3–5 times a day in the light treated infants and 1–3 times a day in the patients without light treatment As seen in Fig 1 a similar decrease in body weight during the first week of life occurred in both groups No skin reaction from the light could be observed Some degree of somnolence in the light treated infants was regularly seen but it could not be determined whether this was due to the treatment or to the fact that the treated infants had higher bilirubin levels than the controls

Table 2 summarizes the results of the analyses in the infants Neither the albumin concentration nor the total protein concentration nor the total protein concentration changed during phototherapy and the electrophoresis pattern remained the same (In one child patient 15 there was a clear decrease in both total protein and albumin No explanation for this could be found)

The albumin–bilirubin binding values which were measured at two different bilirubin concentrations (molar ratio albumin–bilirubin 1 0 5 and 1 1) did not show any statistically significant difference before and after phototherapy

The binding of salicylate to plasma proteins was somewhat variable in all infants including the controls and no significant change during light treatment could be found Again patient number 15 was an exception (see Table 1)

A clear increase in acetylsalicylic acid esterase activity after light treatment was observed (Fig 2) This occurred in most of the light treated infants but in none of the control patients The increase was between 10 and 20%

The hematocrit values in the two groups of infants are shown in Fig 3 In 13 of the 15 light treated infants the hematocrit value increased and in two cases this reaction was especially great In contrast the hematocrit values decreased in the controls

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Acta Pædiatr Scand 64 299–304 1975

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KEY WORDS Hypertension childhood renal disease nephrectomy

Goldblatt et al (11) demonstrated conclusively that bilateral renal ischaemia could produce sustained arterial hypertension in dogs without impairing renal excretory function This finding was confirmed by Wood & Cash (42) who also observed that in some animals persistent hypertension followed unilateral ischaemia produced by a Goldblatt clamp Butler (5) first succeeded in curing hypertension by nephrectomy in a 10-year old girl having accidentally achieved the same result 2 years previously in a 7 year old boy Since the appearance of Butler s paper some hundreds of cases have been reported although unfortunately the value of many of these reports is lessened by the inclusion of insufficient data in the published accounts The resulting difficulties in interpretation led Homer Smith (33) to examine all the available literature on the subject in an attempt to introduce some order into the chaos of conflicting opinions concerning the prognosis of hypertension treated by nephrectomy He reviewed 575 published cases finding that only 26% of these could be accepted as cures according to what he regarded as adequate criteria In the same paper he listed these criteria which are set out below and they have been accepted by most subsequent authors It is interesting that in spite of advances in both surgical and biochemical knowledge reports appearing in recent years show little or no improvement in cure rate over Smith s 26%

The great majority of patients undergoing nephrectomy have been not surprisingly adults in middle or late life Although some writers have commented that young patients seem to do better than older ones in only one paper (31) is an attempt made to justify this opinion with figures and here many of the cases had been followed for too short a time to allow meaningful assessment Of their children however 14 (47%) appear to fulfil

the results of Odell et al (7) in the experiments *in vitro* with illumination of whole blood or plasma There was no change in albumin–bilirubin binding following the 24 hour light treatment This was also true regarding the protein–salicylate binding Thus it seems unlikely that kernicterus could arise from an increase in the unbound fraction of bilirubin during phototherapy

Changes in the circulating blood may be expected as one of the results of light treatment A rise in hematocrit was actually found in the present study Oh & Karecki (8) reported increased stool water loss and insensible water loss during phototherapy An alternative explanation would possibly be a change in distribution ratio between intra and extravascular water The acetylsalicylic acid esterase served as a model for erythrocyte enzyme activity in our experiments A remarkable increase in activity under the influence of light was seen both in the newborn infants in the rabbits and in the *in vitro* experiments with whole blood The possible significance of this finding on salicylate pharmacokinetics should be studied further At the present time it should be seen as a demonstration that enzymes within erythrocytes may be influenced during light irradiation possibly as a result of changes in membrane permeability allowing increased availability of substrates present in the plasma Light induced changes in membrane function might also be an explanation of the increased amounts of unconjugated bilirubin in the bile (10) and in urine (15) after phototherapy The possibility of a light induced rapid stimulation of microsomal and mitochondrial enzymes should also be examined (1)

ACKNOWLEDGEMENT

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REFERENCES

1 Behrman R E & Hsia D Y Y Summary of a symposium on phototherapy for hyperbilirubinemia *J Pediatr 75* 718 1964
2 Brathd D Reserve albumin binding capacity salicylate saturation index and red cell binding of bilirubin in neonatal jaundice *Arch Dis Child* 8 393 1973
3 Cremer R J Perryman P W & Richards D H Influence of light on the hyperbilirubinemia in infants *Lancet I* 1094 1958
4 Lucey J F Ferreiro M & Hewitt J Prevention of hyperbilirubinemia of prematurity by phototherapy *Pediatrics 41* 1047 1968
5 Lucey J F Neonatal phototherapy use problems and questions *Semin Hematol 9* 127 1972
6 Maisels M J Bilirubin on understanding and influencing its metabolism in the newborn infant *Pediatr Clin North Am 19* 447 1972
7 Odell G B Brown R S & Holzman N A Dye-sensitized photo-oxidation of albumin associated with a decreased capacity for protein binding of bilirubin *In* Bergsma D (ed) Bilirubin metabolism in the newborn *Birth Defects 6* 31 1970
8 Oh W & Karecki H Phototherapy and insensible water loss in the newborn infant *Am J Dis Child 124* 230 1972
9 Ostrow J D Photocatabolism of labeled bilirubin in the congenitally jaundiced (Gunn) rat *J Clin Invest 50* 707 1971
10 — Mechanism of bilirubin photodegradation *Semin Hematol 9* 113 1972
11 Ostrow J D & Branham R V Photodecay of bilirubin in vitro and in the jaundiced (Gunn) rat *In* Bergsma D (Ed) Bilirubin metabolism in the newborn *Birth Defects 6* 93 1970
12 Porto S O Pildes R S & Goodman H Studies on the effect of phototherapy on neonatal hyperbilirubinemia among low birth weight infants II Protein binding capacity *J Pediatr 75* 1048 1969
13 Potter G B & Guy J L A micro method for analysis of plasma salicylates *Proc Soc Exp Biol Med 116* 658 1965
14 Thaler M M & Schmid R Drug and bilirubin *Pediatrics 47* 807 1971
15 Weiss P A M & Rosanelli K Quantitative chromatogrammspektralphotometrische Bestimmungen von freiem Bilirubin aus dem Harn von Säuglingen nach Phototherapie sowie aus Fruchtwasser bei rhesusinkompatiblen Schwangerschaften *Wien Klin Wochenschr 84* 671 1972
16 Windorfer A Jr & Mihailova K Der Einfluss verschiedener Medikamente und pH Werte auf die Albumin–Bilirubin Bindung in vitro *Z Kinderheilkd 114* 83 1973
17 Windorfer A Jr Mihailova K & Pringsheim W Besteht erhöhte Gefahr einer Bilirubinencephalopathie bei medikamentöser Therapie im Neugeborenenalter? *Dtsch Med Wochenschr 98* 1 08 1973

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Smith s criteria and an additional 6 (20%) are possible cures

This paper reports 2 further successful cases and examines the results of the 45 cases in the literature under 15 years of age at the time of operation which were reported with sufficient information to permit proper assessment after a minimum follow up period of one year

CASE REPORTS

Case 1

A 10-year-old girl presented with a 1 year history of morning headaches nausea and vomiting worsening over the last few weeks There were no other symptoms and in particular no symptoms related to the urinary tract Examination revealed a blood pressure in the range 160/100–190/120 over a period of several days Grade I hypertensive retinopathy was present there were no other abnormalities

Laboratory investigations Blood urea 19 mg/100 ml Plasma sodium 143 mEq/l potassium 3 6 mEq/l chloride 96 mEq/l bicarbonate 32 mEq/l Urine microscopy no formed elements seen urine sterile to culture Urinary VMA excretion not increased

Radiology Intravenous urography showed a very small poorly functioning distorted kidney on the right the left appeared normal but was larger than average for age Retrograde pyelography showed marked calyceal clubbing and cortical thinning on the right the appearances being typical of chronic pyelonephritis

Cystoscopic appearances were normal Right nephrectomy was performed and the blood pressure fell rapidly to 100/60 in the post-operative period After 4 years she remained perfectly well with creatinine clearance 62 ml/min/m² and a blood pressure of 110/65 After 5 years blood pressure remained normal and intravenous urography showed the left kidney to be normal and to show compensatory hypertrophy Histological examination of the removed kidney showed it to be shrunken and scarred the appearances being consistent with chronic pyelonephritis although whether the kidney was hypoplastic could not be stated with confidence

Case 2

A girl aged 5 years 10 months presented with a 6 week history of morning headache and vomiting accompanied by slight mental confusion Her blood pressure was 220/180 and the optic fundi showed early papillo-edema and one exudate Nothing in history or examination was suggestive of renal or urinary tract disease

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Radiology showed a small scarred and shrunken right kidney the appearances being consistent with chronic pyelonephritis The left kidney was large for age but otherwise normal

Intravenous phentolamine test was within normal limits. Right nephrectomy was performed and the blood pressure was normal within 4 days Her blood pressure 1 year after operation was 105/75 and remained normal for a further ? years of follow up 4 months post operatively the creatinin clearance was 35 and 42 ml/min/m² (lower limit of normal in this laboratory 50 ml/min/m²) and the blood urea ?8 mg/100 ml Histology of the removed kidney confirmed that it was extremely small and shrunken As in case 1 no definite opinion could be given as to whether the kidney was a hypoplastic one with secondary pyelonephritis or whether the organ had been destroyed by pyelonephritis

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In order for a case to be accepted as a cure Smith demanded that it fulfil the following requirements

(1) Definitive hypertension must be established pre operatively and not predicated simply on one or a few blood pressure determinations

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Clearly the second of these criteria includes an unsatisfactory definition of the normal range of blood pressure when applied to children An acceptable substitute for the figure 140/90 would seem to be less than 2 standard deviations above the mean value for age and sex the relevant data are available from the literature (19 22) 140/90 is in fact approximately 2 S D above the mean at puberty in both sexes so this modification agrees well in principle with Smith s original intention A search of the literature has revealed only 45 children under the age of 15 years at the time of operation who were reported in a manner complete enough to allow the application of these criteria and the important data from these cases are summarised in Table 1

Thus 34 (76%) were cured 9 (20%) were failures i e alive but not normotensive post operatively 1 (2%) died in the early post operative period and 1 (2%) had a borderline normal blood pressure at 1 year These results are strikingly better than those obtained in

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Died of unrelated causes

adults even though the methods used in selection and treatment of cases were essentially the same in the 2 groups It may be objected that both the adult and the childhood series suffer from under reporting of cases in that many more of these operations have probably been done than have reached the literature This is undoubtedly true but there is no reason to suppose that the childhood group is either more or less biased than the adult group particularly in view of the fact that with a few exceptions (6 7 12 14 17 20 24 34 39 41) the children in the table were extracted from the same group of reports which together constitute the total series

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ing out of this condition is the selection of cases suitable for operation The children in the reported series together with the two newly reported cases have certain important features in common where treatment was successful *Firstly* all had gross disease of one kidney readily recognisable by simple investigations The most common stated diagnosis is pyelonephritis accounting for 23 cases (52%) although it is probable that some of these including perhaps the two new cases described here were really dysplastic or hypoplastic kidneys masquerading as chronic atrophic pyelonephritis the distinction between these two conditions is always difficult and may be impossible on either radiological or histological grounds Whatever the nature of the underlying disease however it was without exception evident on simple radiology of the renal tract i e by a combination of urography pyelography and arteriography *Secondly* in all the successful cases the other kidney was radiologically normal and in most cases compensatory hypertrophy was present before nephrectomy In this paper only children with unilateral disease have been considered since in all reported cases in which the preserved kidney was known to be diseased before operation the procedure was not effective in relieving the hypertension for example in one report (41) 6 of 7 children with unilateral disease were cured while all of 4 with known bilateral pyelonephritis failed even though the changes in the less affected kidney were mild in each case

An interesting feature in the 2 children now reported is the abnormality of the plasma electrolytes which in case 2 in particular suggests aldosteronism It is likely that this is due to activation of the renin hypertension system and it is tempting to attribute the hypertension in this situation to increased renin production by the diseased kidney Various workers have attempted to refine the selection of cases for operation by measuring peripheral venous blood renin levels or more recently bilateral renal vein renin levels (2 38) This technique appears to be of some value but suffers from the limitation that a negative result i e failure to demonstrate a raised level does not exonerate the suspect kidney as the cause of the hypertension This is probably due to two things (*a*) although quite a good correlation exists between unilateral elevation of renal vein renin and hypertension due to *renovascular* hypertension there is no good evidence of such a relationship where the renal disease is *parenchymal* in nature (*b*) in experimental animals hypertension due to renal ischaemia is associated with high blood renin levels in the early stages of the condition but after a time the renin levels may fall even though the hypertension persists (9) Thus it may be that renin plays a role in initiating but not in sustaining renovascular hypertension The paper of Arakawa et al (2) argues an additional advantage in measuring renal vein and peripheral blood renin in that if the level in the renal vein draining the supposedly normal kidney is higher than that in peripheral blood it is likely that the kidney is contributing to the maintenance of the hypertension in which case operation would be unlikely to be of benefit This interesting suggestion warrants further investigation and may prove to be the means of avoiding an ineffective nephrectomy in some patients At present however the value of blood renin levels in assessing renal hypertension is uncertain and in cases such as those described here where a grossly shrunken and abnormal kidney on one side was accompanied by radiological evidence of compensatory hypertrophy in the other it is doubtful whether the information gained would justify the additional small risk of the invasive procedure of bilateral renal vein catheterisation

An alternative method of incriminating a kidney as the cause of arterial hypertension is the performance of split renal function tests involving bilateral ureteric catheterisation and measurements of urine flow concentration and clearance of creatinine and *p* amino hippurate (PAH) and sodium excretion (16 35 36) Once again in practice the predictive value of these

tests falls short of theoretical expectations and no clear relationship between a particular pattern of abnormality and the likelihood of surgical cure has been demonstrated Dunstan et al (8) found that as in the case of renin measurements there was some correlation in the case of primary renovascular disease but none in parenchymal disease Stewart et al (37) report similar findings in 24 patients with chronic pyelonephritis they found no positive split renal function tests but of the 6 who were treated by nephrectomy 4 derived benefit from the procedure Here again it may be that the principal value of split function studies is confirmation of normality in the contralateral kidney criteria for which were laid down by Stamey (36)

A probable factor in the causation of renal hypertension is derangement of sodium and water excretion with sodium retention leading to expansion of extracellular volume and systemic hypertension This might occur in at least two ways one by the production of a hormone such as aldosterone which on release into the general circulation will lead to sodium retention by *both* kidneys two by an intrinsic neural or hormonal link between the juxta glomerular apparatus and the tubule within the *individual* kidney not subject to systemic hormonal or other extrinsic influences Of these two mechanisms the first may well operate in the case of unilateral disease and the electrolyte pattern in the 2 children here described is consistent with aldosteronism (hypokalaemia and metabolic alkalosis) The second does not appear relevant in strictly unilateral disease since one would expect any tendency of the diseased kidney to retain sodium and water by intrinsic mechanisms to be nullified by the healthy kidney as soon as significant expansion of the extracellular volume occurred

It remains the case that in most cases of hypertension associated with unilateral renal disease the nature of the mechanisms involved is obscure A rational prediction of which cases are likely to benefit from surgery is therefore difficult It is unlikely that further special investigation will help in children such as the two presented here with (*a*) gross disease with loss of substance of one kidney (*b*) evidence of compensatory hypertrophy of the other kidney and (*c*) no evidence of impaired excretory function Where doubt exists about the normality of the contralateral kidney then either failure of suppression of renal vein renin on the side of the supposedly normal kidney or evidence of impaired function on split ureteric studies would suggest that nephrectomy would not relieve the hypertension and should not be advised This is an important consideration since nephrectomy for unilateral parenchymal renal disease is uniformly unsuccessful in relieving hypertension if there is evidence even of mild abnormality of the contralateral kidney

The findings summarised in this paper suggest that in children of less than 15 years of age presenting with hypertension associated with gross unilateral kidney disease removal of the diseased kidney produces a cure in about three quarters and that the age of the patient is one of the most important factors in the prognosis of this condition Identification of cases suitable for nephrectomy may be difficult and both renal vein renin estimations and split renal function studies may be required to substantiate the normality of the preserved kidney rather than to add useful information about the diseased kidney

REFERENCES

1 Abeshouse B S Hypertension and unilateral renal disease Review of the literature and report of 16 cases *Surgery 9* 942 *1941*

2 Arakawa K Masaki Z Osada Y Yamada J Momose S Divided renal and peripheral venous renin as a means of predicting operative curability of renal hypertension *Clin Sci 45* 311 1973

3 Barney J D & Suby H I Unilateral renal disease with arterial hypertension *N Engl J Med 220* 744 1939

4 Benjamin B & Ratner M Hypertension associated with unilateral chronic atrophic pyelonephritis *Am J Dis Child 61* 1051 1941

5 Butler A M Chronic pyelonephritis and arterial hypertension *J Clin Invest 16* 889 1937

ing out of this condition is the selection of cases suitable for operation The children in the reported series together with the two newly reported cases have certain important features in common where treatment was successful *Firstly* all had gross disease of one kidney readily recognisable by simple investigations The most common stated diagnosis is pyelonephritis accounting for 23 cases (52%), although it is probable that some of these including perhaps the two new cases described here were really dysplastic or hypoplastic kidneys masquerading as chronic atrophic pyelonephritis the distinction between these two conditions is always difficult and may be impossible on either radiological or histological grounds Whatever the nature of the underlying disease however, it was without exception evident on simple radiology of the renal tract i e by a combination of urography pyelography and arteriography *Secondly* in all the successful cases the other kidney was radiologically normal and in most cases compensatory hypertrophy was present before nephrectomy In this paper only children with unilateral disease have been considered since in all reported cases in which the preserved kidney was known to be diseased before operation the procedure was not effective in relieving the hypertension for example in one report (41) 6 of 7 children with unilateral disease were cured while all of 4 with known bilateral pyelonephritis failed even though the changes in the less affected kidney were mild in each case

An interesting feature in the 2 children now reported is the abnormality of the plasma electrolytes which in case 2 in particular suggests aldosteronism It is likely that this is due to activation of the renin hypertension system and it is tempting to attribute the hypertension in this situation to increased renin production by the diseased kidney Various workers have attempted to refine the selection of cases for operation by measuring peripheral venous blood renin levels or more recently bilateral renal vein renin levels (2 38) This technique appears to be of some value but suffers from the limitation that a negative result i e failure to demonstrate a raised level does not exonerate the suspect kidney as the cause of the hypertension This is probably due to two things (*a*) although quite a good correlation exists between unilateral elevation of renal vein renin and hypertension due to *renovascular* hypertension there is no good evidence of such a relationship where the renal disease is *parenchymal* in nature (*b*) in experimental animals hypertension due to renal ischaemia is associated with high blood renin levels in the early stages of the condition but after a time the renin levels may fall even though the hypertension persists (9) Thus it may be that renin plays a role in initiating but not in sustaining renovascular hypertension The paper of Arakawa et al (2) argues an additional advantage in measuring renal vein and peripheral blood renin in that if the level in the renal vein draining the supposedly normal kidney is higher than that in peripheral blood it is likely that the kidney is contributing to the maintenance of the hypertension in which case operation would be unlikely to be of benefit This interesting suggestion warrants further investigation and may prove to be the means of avoiding an ineffective nephrectomy in some patients At present however the value of blood renin levels in assessing renal hypertension is uncertain and in cases such as those described here where a grossly shrunken and abnormal kidney on one side was accompanied by radiological evidence of compensatory hypertrophy in the other it is doubtful whether the information gained would justify the additional small risk of the invasive procedure of bilateral renal vein catheterisation

An alternative method of incriminating a kidney as the cause of arterial hypertension is the performance of split renal function tests involving bilateral ureteric catheterisation and measurements of urine flow concentration and clearance of creatinine and *p* amino hippurate (PAH) and sodium excretion (16 35 36) Once again in practice the predictive value of these

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EXCHANGE TRANSFUSIONS WITH CONCENTRATED ACD BLOOD

I *Effects on the Infant s Red Cell Volume Plasma Volume and Haemoglobin Mass*

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ABSTRACT Kreuger A and Wranne L (Department of Paediatrics and Blood Center University Hospital Uppsala Sweden) Exchange transfusions with concentrated ACD-blood I Effects on the infant s red cell volume plasma volume and haemoglobin mass Acta Paediatr Scand 64 305 1975.—During ten exchange transfusions the infant s gain or loss of haemoglobin and of the volumes of red cells and plasma were studied quantitatively The gain or loss of haemoglobin was found to be closely correlated to the difference between the haemoglobin concentration of the given blood and that of the infant before the exchange transfusion The standard ACD blood used was hypo-osmolar (NIH sol B) and its red cells overhydrated During the exchange the cells returned to normal and released water about 10 ml per kg of the infant s bodyweight Haematocrit readings therefore are unsuitable for the judgement of the ACD donor blood s oxygen transport capacity As a rule less plasma was given than removed However if the above-mentioned water released from the red cells was included in the given volume the latter became almost equal to the volume removed

KEY WORDS Exchange transfusions newborn

Exchange transfusion (ET) as a treatment of haemolytic disease and hyperbilirubinaemia in the newborn has become a routine procedure Little attention seems to have been paid to the total amounts of plasma haemoglobin and red cells in the given and removed blood considering the widespread use of ETs In fact we could find no such studies in the literature The following is a report of a study on ten exchange transfusion

MATERIAL AND METHODS

Patients Nine newborn infants were studied during ten ETs In 4 infants the ET was performed because of hyperbilirubinaemia without known cause the remaining infants had haemolytic disease due to Rh immunization of their mothers (Table 1) No infant was seriously anaemic

Donor blood Concentrated ACD-blood not older than 96 hours was used To each 400 ml of donor blood 100 ml of ACD solution (NIH sol B) was added These 100 ml contain 1 32 g sodium citrate 0 48 g citric acid and 1 47 g glucose Before use 1 5 ml of the plasma ACD mixture was removed and discarded

Measurement of the administered blood The concentrated ACD blood was carefully mixed and 20 ml removed from each bottle for analysis The blood content of each bottle was measured by weighing the bottle before and after ET Blood remaining in the tubing at the end of the ET was siphoned back into the bottles However in spite of this 5–15 ml of donor blood usually remained in the tubing This was removed by rinsing the tubing with a known amount of ion free water The oxy haemoglobin concentration of the haemolysate thus obtained was measured spectrophotometrically and compared with that of a suitable dilution of the blood from the same donor bottle The volume of the blood remaining in the tubing could then be calculated Careful notes were kept on other losses of donor blood to ensure a proper estimation of the amount of administered blood The conversion of weighed amounts of blood to volumes was made after determination of specific weight by weighing volumetric flasks filled with the donor blood

Measurement of the removed blood The removed blood was collected in a glass bottle Coagulation was prevented by the addition of 10 000 units heparin to the collecting

6 Coran A G & Schuster S R Renovascular hypertension in childhood *Surgery 64* 672 1968
7 Douglas D M Lowe K G & Mitchell R G Hypertension and unilateral renal disease treated by nephrectomy *Br Heart J 21* 361 1959
8 Dunstan H P Page I H Poutasse F F & Wilson L Treatment of hypertension associated with occlusive renal arterial disease *Circulation 27* 1018 1963
9 Fraley E E & Feldman B H Renal hypertension *N Engl J Med 287* 550 1972
10 Gasul B M Glasser J M & Grossman A Extreme hypertension in a child cured by nephrectomy *JAMA 139* 305 1949
11 Goldblatt H Lynch J Hanzal R F & Summerville V W Studies in experimental hypertension I The production of persistent elevation of B P by means of renal ischaemia *J Exp Med 59* 347 1934
12 Grant P Unilateral renal disease with hypertension in children surgical aspects *Med J Aust II* 814 1960
13 Griffiths A L Hypertension of renal origin in children *Arch Dis Child 25* 81 1950
14 Harnaes Kr & Seip M Unilateral kidney disease with hypertension in children *Acta Paediatr Scand 49* 28 1960
15 Higbee D R Congenital renal hypoplasia associated with hypertension report of 2 cases *J Urol 51* 466 1944
16 Howard J E & Connor T B Use of differential renal function studies in the diagnosis of renovascular hypertension *Am J Surg 107* 58 1964
17 Isaacson C & Wayburne S Malignant hypertension in a child due to unilateral renal disease *Arch Dis Child 32* 106 1957
18 Kennedy R L J Barker N W & Walters W Malignant hypertension cure following nephrectomy *Am J Dis Child 69* 160 1945
19 Londe S Blood pressure in children as determined under office conditions *Clin Pediatr (Phila) 5* 71 1966
20 Luke R G Kennedy A C Briggs J D Struthers N W Watt J K Short D W & Barr Stirling W Results of surgery in hypertension due to renal artery stenosis *Br Med J II* 76 1968
21 Mathison H S Arterial hypertension in unilateral renal disease with inhibition of growth treated with nephrectomy *Acta Med Scand 139* 421 1951
22 Moss A J Liebling W & Adams F The flush method for determining blood pressures in infants *Pediatr 21* 950 1958
23 Movin R Ohlsen A S & Pederson A M Arterial hypertension—nephrectomy *Acta Med Scand 119* 439 1944
24 Nennhaus H P Javid H & Hunter J A Surgical treatment of renovascular hypertension in children with a review of infradiaphragmatic arterial hypoplastic anomalies *J Thorac Cardiovasc Surg 54* 246 1967
25 Oculicz S J & Marshall V F Nephrectomy and hypertension *Am J Surg 86* 45 1953
26 Øster J Arterial hypertension in a child cured by nephrectomy *Acta Med Scand 128* 42 1947
27 Peart W S Results of medical versus surgical treatment of renovascular hypertension *Clin Sci 45* 89 1973
28 Perkel H Acute hypertension probably secondary to infarctions within a congenitally hypoplastic kidney *J Pediatr 36* 254 1950
29 Perloff D Sokolow M Wylie E J Smith D R & Palubinskas A J Hypertension secondary to renal artery occlusive disease *Circulation 24* 1286 1961
30 Pickering G W & Heptinstall R H Nephrectomy and other treatment for hypertension in pyelonephritis *Q J Med NS XXII* 1 1953
31 Schaffer A J & Markowitz M Hypertension treated by nephrectomy a report of 4 additional cases and a re-evaluation of prognosis and criteria for operation *Am J Med Sci 227* 417 1954
32 Semans J H Nephrectomy for hypertension in a 3½ year old child with apparent cure for 3 years *Bulletin of the Johns Hopkins Hospital 75* 184 1944
33 Smith H W Unilateral nephrectomy in hypertensive disease *J Urol 76* 685 1956
34 Smith J P & Saylor M L Renal vascular hypertension in children *J Urol 84* 17 1960
35 Stamey T A Nudelman I J Good P H Schwentker F N & Hendricks F Functional characteristics of renovascular hypertension *Medicine 40* 347 1961
36 Stamey T A In F Gross (ed) *Antihypertensive Therapy (Principles & Practice)* Springer Verlag Berlin 1966 p 555
37 Stewart B H Schacht R A Bishop R C & Conway J Differential function studies in renal hypertension indications and techniques *J Urol 94* 7 1965
38 Stockigt J R Collins R D Noakes C A Schambelan M & Biglieri E G Renal vein renin in various forms of renal hypertension *Lancet I* 1194 1972
39 Ullmann T D Shapiro T Barzilay B & Schwartz A Abnormalities in a well functioning kidney as the cause of long standing severe juvenile hypertension cured by unilateral nephrectomy *Am J Med 26* 960 1959
40 Van Goidsenhoven F & Vandenbrouk J Formes curables de l hypertension renale chirurgicale ou urologique *Presse Med 54* 52 1946
41 Welch H C Harris L E & de Weerd H Nephrectomy in juvenile hypertension associated with renal disease *Pediatr 21* 941 1958
42 Wood J E Jr & Cash J R Experimental hypertension—observations on sustained elevation of systolic and diastolic blood pressure in dogs *J Clin Invest 15* 543 1936

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Table 3 *The concentrations of haemoglobin the haematocrit and the MCHC in venous blood from the infants before and after the exchange and of the given and removed blood*

Mean values and S D of ten exchange transfusions

	Infant before	Given blood	Removed blood	Infant after
Hb concentration g/l	166± 0	151±7	158± 9	156± 8
Hct volume fraction	0 515±0 060	0 538±0 034	0 500±0 0 6	0 485±0 015
MCHC concentration g/l	3??±15	?81±7	316±14	3 0±10

blood and of the infants blood before and after the ET are shown in Table 3 No infant was seriously anaemic the mean venous haematocrit before the ET was 0 515 (range 0 425–0 600) and the mean haemoglobin concentration before the ET was 166 g/l (range 136–190 g/l)

Red cell volume and total haemoglobin mass (Table 4 Fig 1)

The infants RCV per kg bodyweight before the ET are shown in Table 4 together with the RCV of the volumes of given and removed blood On the average the infants RCV was 40 2 ml/kg before the ET 91 8 ml/kg were given and 86 9 ml/kg were removed In the same Table 4 the THb per kg bodyweight of the infants before the ET and the THb in given and removed blood are shown On the average the infants had 12 9 g/kg before ET 25 8 g were given and 27 4 g/kg removed From these three estimates of THb the THb of the infants after the ET could be deducted It averaged 11 3 g/kg Consequently knowing the infants MCHC after the ET their RCV after the ET could be estimated to be on average 35 3 ml/kg It then became obvious that the given red cells must have lost fluid to the infants plasma during the ET This volume of fluid averaged 9 8 ml/kg and the mean differed significantly from zero ($p<0\ 001$)

Plasma volume

The plasma volumes (PV) of the infants before and after the ET were calculated as described above In Table 4 they are shown together with the given and removed amounts of plasma The infants mean PV per kg bodyweight before and after the ET were 46 1 and 45 6 ml respectively and the given and removed volumes 78 8 and 87 0 ml respectively The red cells however lost fluid during the ET on the average 9 8 ml/kg bodyweight Accordingly the given volume of plasma fluid should be estimated to 78 8+9 8=88 6 ml/kg which is 1 6 ml more than the removed volume The infants mean PV decreased by 46 1−45 6=0 5 ml/kg during the ET which implies the insignificant outflow of 1 6+0 5=2 1 ml to the extravascular space

DISCUSSION

The present study was undertaken in an effort to obtain an answer to the question whether an

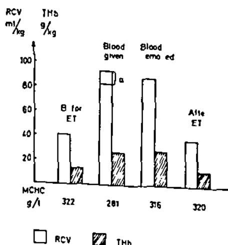


Fig 1 Red cell volume (RCV) total haemoglobin mass (THb) and the mean corpuscular haemoglobin concentration before and after the ET and in the given and removed blood (*a*)=fluid transferred from the red cells to the plasma

Table 1 *Some clinical data*

HDN Rh=haemolytic disease of the newborn due to Rh immunization of the mother Hyperbil =hyperbilirubinaemia of the newborn without diagnosed immunization of the mother

No	Gestational age (weeks)	Weight at ET (kg)	Age at onset of ET (hours)	Ordinal no of ET / Total no of ET	Diagnosis
1	35	1 95	122	1/1	Hyperbil
2	32	2 02	4	2/5	HDN Rh
3	32	2 02	15	4/5	HDN Rh
4	35	2 05	127	2/2	Hyperbil
5	35	2 16	0 5	1/1	HDN Rh
6	33	2 17	78	1/1	Hyperbil
7	36	2 24	143	1/2	Hyperbil
8	37	2 80	3	1/2	HDN Rh
9	42	3 33	4	1/1	HDN Rh
10	40	3 58	3	1/1	HDN Rh

bottle and by shortening the waste tube as much as possible The blood was weighed and its volume was calculated from standard tables for blood of varying haemoglobin and protein concentrations (4)

Analyses Haematocrit readings were obtained by a high speed micro haematocrit centrifuge (International) No correction for trapped plasma was made Haemoglobin determinations were made by the cyanomethaemoglobin method and checked by commercially available standards These measurements were made on each bottle of donor blood on the removed blood and on the blood from the infant before and after the ET

Calculations of the infant s blood volume Calculations of the infant s pre exchange total volumes of blood plasma (PV) and red cells (RCV) were performed according to Bratteby i e RCV in ml/kg bodyweight=102 venous haematocrit − 12 3 (3) Assuming that the mean corpuscular haemoglobin concentration (MCHC) was the same in body blood as in venous blood their total haemoglobin mass (THb) could also be derived The infants postexchange volumes could not be calculated in the same manner however The post exchange THb was obtained by adding the pre-exchange THb to the given THb and subtracting the removed THb (Table 4) The post-exchange THb and MCHC then gave the post exchange RCV (Table 4) The post exchange PV was calculated from the last mentioned RCV and the post exchange haematocrit assuming that the body haematocrit/venous haematocrit ratio was unchanged by the ET The ratio used was 0 90 (2)

Exchange transfusion technique In the standard procedure 170 ml of blood per kg body weight was given and 5 ml removed in excess of the volume administered The ET was performed in 10–20 ml portions and was completed in about 60 minutes Albumin or calcium salts were not given The temperature of the donor blood was kept at 36°C by a heat-exchanger

Gain and loss calculation A gain and loss account could be made by comparing the amounts and constituents of the given blood with those of the removed blood Blood samples taken from the infants for analyses were included in the account

Table 2 *The volumes of whole blood red cells and plasma given and removed from the infants*

All values are expressed per kg body weight mean values and S D of ten exchange transfusions

	Given volume	Removed volume
Whole blood ml/kg	170 6±9 8	173 9±7 8
Red cells ml/kg	91 8±9 5	86 9±6 4
Plasma ml/kg	78 8±5 6	87 0±5 6

RESULTS

Mean values of the given volumes and the removed volumes of whole blood red cells and plasma are shown in Table 2 On the average 3 3 ml blood was removed per kg bodyweight in excess of the volume given The red cell volume of the given blood was 4 9 ml/kg greater than that of the removed Thus the plasma volume of the given blood was 8 2 ml/kg less than that of the removed blood

The difference between the given and the removed amount of blood was not the same at every ET The largest excess of blood given was 7 4 ml/kg and the largest deficit created was 15 6 ml/kg

The mean concentrations of haemoglobin and haematocrit of the given and removed

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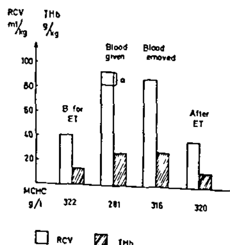


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Table 4 *Total haemoglobin mass (THb) and the volumes of red cells and plasma of the infants before and after the exchange and of the given and removed blood*

All values are expressed per kg body weight mean values and S D of ten exchange transfusions The right hand column shows the fluid transferred from the red cells to the plasma

	Infant before *a*	Given *b*	Removed *c*	Infant after *d*	*a+b−c−d*
THb g/kg	12 9±2 0	25 8±2 8	27 4±1 5	11 3±1 6	
Red cell volume ml/kg	40 2±6 2	91 8±9 5	86 9±6 4	35 3±5 8	9 8±3 1
Plasma volume ml/kg	46 1±3 1	78 8±5 6	87 0±5 6	45 6±7 3	

infant gains or loses blood constituents during an exchange transfusion This question although seeming simple could not be answered by a study of the literature since earlier studies reported only the concentrations of various blood constituents in given and removed blood In the present report the volumes of red cells and of plasma and the amounts of haemoglobin gained or lost have been investigated

The prediction of the infant s RCV from the venous haematocrit was performed according to Brattebý who has pointed out that this should be valid for normal full term infants as well as for prematures (3) The body haematocrit/venous haematocrit ratio of 0 90 is an approximated value but agrees with ratios found in newborns as well as adults (2) Thus the blood volumes could be calculated in the same manner for all infants in the present investigation though the group consisted of both prematures and full term infants

The main clinical interest is directed to differences between blood constituents given and removed Therefore volumes and concentrations of given and removed blood were measured in duplicate During an ET there is a risk of overloading the infant s circulation Consequently at the end of the ET the child was routinely left with a calculated deficit of about 5 ml blood per kg bodyweight In spite of this there was a difference between the given and removed amounts of blood per kg bodyweight in our series ranging from an excess of 7 4 ml to a deficit of 15 6 ml at the end of the ET These differences should be ascribed to inevitable errors in the routine measurements of blood volumes in the syringes during the ET

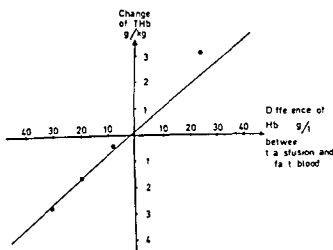


Fig 2 The change in the total haemoglobin mass as a function of the difference of the blood Hb concentration between the transfusion blood and the infant before ET Correlation coefficient=0 95

Haemoglobin (Tables 3 and 4 Fig 2)

Concentrated blood was used for the ET The infants mean haemoglobin concentration was lowered slightly by the ET On the average 1 6 g Hb per kg bodyweight was lost which corresponds to more than 10% of the infants mean pre exchange total haemoglobin mass The loss was not significant However infants with anaemia would have gained haemoglobin by the ET

The infants loss or gain of haemoglobin could be further studied by plotting the change of the individual infant s total haemoglobin mass (THb) as a function of the difference between the haemoglobin concentration of the given blood and that of the infant before the ET

Fig 2) As could be expected there was a close correlation a higher haemoglobin concentration in donor blood than in the infant s blood gave a gain of haemoglobin and vice versa ($r=0.95$) By removing more or less plasma from the ACD donor blood before the ET the infant s gain or loss of haemoglobin may be regulated

Red-cell volume (Tables 3 and 4 Fig 1)

The mean haematocrit (hct) of given blood was higher than that of the infant In removed blood mean hct was lower than that of the infants before the ET and after the ET the infants had still lower mean hct These seemingly conflicting results were explained by a study of the mean corpuscular haemoglobin concentration (MCHC) which was low in the red cells of the given blood This finding is characteristic of standard ACD-donor blood (NIH sol B) owing to its hypo-osmolarity (5) The given red cells thus were overhydrated but seemed to shrink during the ET In removed blood as well as in the infants before and after the ET the MCHC s were normal The volume of fluid released from the red cells during the ET could be calculated to 9 8 ml/kg on the average corresponding to about 25% of the infant s calculated pre exchange plasma volume Consequently when using a hypo osmolar blood preservative the haematocrit of the given blood should not be assumed to bear a direct relationship to its total haemoglobin and oxygen transporting capacity Thus the use of a hypo-osmolar blood preservative for the ET blood comprises a risk of diminishing the total haemoglobin of the infant during the ET (Table 4) The risk is enhanced if the infant is left with a blood deficit after the ET

Plasma volume (Table 4)

A larger amount of plasma was removed than given this was however compensated for by the above mentioned release of fluid from the red cells The infants mean plasma volume after the ET could also be calculated and agreed well with that obtained by the gain and loss account (Table 4) indicating that no significant net transfer of plasma water had occurred to the extravascular space

REFERENCES

1 Allen F H & Diamond L K *Erythroblastosis fetalis* Little Brown & Co Boston and Toronto 1957
2 Bratteby L E Studies on erythrokinetics in infancy VIII Mixing disappearance rates and distribution volume of labelled erythrocytes and plasma proteins in early infancy *Acta Soc Med Upsal 72* 243 1967
3 — Studies on erythrokinetics in infancy IX Prediction of red cell volume from venous haematocrit in early infancy *Acta Paediatr Scand 57* 125 1968
4 Diem K *Wissenschaftliche Tabellen* J R Geigy A G Basel 1960 6th ed p 517
5 Mollison P L *Blood transfusion in clinical medicine* Blackwell Scientific Publications Oxford London Edinburgh and Melbourne 1972 5th ed p 59

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All values are expressed per kg body weight: mean values and S.D. of ten exchange transfusions. The right hand column shows the fluid transferred from the red cells to the plasma

	Infant before *a*	Given *b*	Removed *c*	Infant after *d*	*a+b−c−d*
THb g/kg	12.9±2.0	25.8±2.8	27.4±1.5	11.3±1.6	
Red cell volume ml/kg	40.2±6.2	91.8±9.5	86.9±6.4	35.3±5.8	9.8±3.1
Plasma volume ml/kg	46.1±3.1	78.8±5.6	87.0±5.6	45.6±7.3	

infant gains or loses blood constituents during an exchange transfusion. This question although seeming simple could not be answered by a study of the literature since earlier studies reported only the concentrations of various blood constituents in given and removed blood. In the present report the volumes of red cells and of plasma and the amounts of haemoglobin gained or lost have been investigated.

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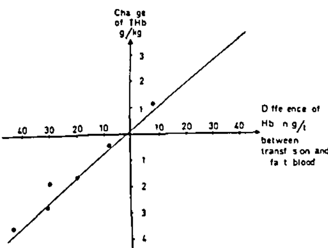


Fig. 2. The change in the total haemoglobin mass as a function of the difference of the blood Hb concentration between the transfusion blood and the infant before ET. Correlation coefficient = 0.95.

Haemoglobin (Tables 3 and 4, Fig. 2)

Concentrated blood was used for the ET. The infants' mean haemoglobin concentration was lowered slightly by the ET. On the average 1.6 g Hb per kg bodyweight was lost which corresponds to more than 10% of the infants' mean pre-exchange total haemoglobin mass. The loss was not significant. However, infants with anaemia would have gained haemoglobin by the ET.

The infants' loss or gain of haemoglobin could be further studied by plotting the change of the individual infant's total haemoglobin mass (THb) as a function of the difference between the haemoglobin concentration of the given blood and that of the infant before the ET

(Fig 2) As could be expected there was a close correlation a higher haemoglobin con centration in donor blood than in the infant s blood gave a gain of haemoglobin and vice versa ($r=0\ 95$) By removing more or less plasma from the ACD donor blood before the ET the infant s gain or loss of haemoglobin may be regulated

Red-cell volume (Tables 3 and 4 Fig 1)

The mean haematocrit (hct) of given blood was higher than that of the infant In removed blood mean hct was lower than that of the infants before the ET and after the ET the infants had still lower mean hct These seem ingly conflicting results were explained by a study of the mean corpuscular haemoglobin concentration (MCHC) which was low in the red cells of the given blood This finding is characteristic of standard ACD donor blood (NIH sol B) owing to its hypo osmolarity (5) The given red cells thus were overhydrated but seemed to shrink during the ET In removed blood as well as in the infants before and after the ET the MCHC s were normal The volume of fluid released from the red cells during the ET could be calculated to 9 8 ml/kg on the average corresponding to about 25% of the infant s calculated pre-exchange plasma vol ume Consequently when using a hypo osmolar blood preservative the haematocrit of the given blood should not be assumed to bear a direct relationship to its total haemoglobin and oxygen transporting capacity Thus the use of a hypo-osmolar blood preservative for the ET blood comprises a risk of diminishing the total haemoglobin of the infant during the ET (Table 4) The risk is enhanced if the infant is left with a blood deficit after the ET

Plasma volume (Table 4)

A larger amount of plasma was removed than given this was however compensated for by the above mentioned release of fluid from the red cells The infants mean plasma volume after the ET could also be calculated and agreed well with that obtained by the gain and loss account (Table 4) indicating that no significant net transfer of plasma water had occurred to the extravascular space

REFERENCES

1 Allen F H & Diamond L K *Erythroblastosis fetalis* Little Brown & Co Boston and Toronto 1957
Bratteby L E Studies on erythrokinetics in infancy VIII Mixing disappearance rat s and distribution vol ume of labelled erythrocytes and plasma proteins in early infancy *Acta Soc Med Upsal 72* 243 1967
3 — Studies on erythrokinetics in infancy IX Prediction of red cell volume from venous haematocrit in early infancy *Acta Paediatr Scand 57* 125 1968
4 Diem K *Wissenschaftliche Tabellen* J R Geigy A G Basel 1960 6th ed p 517
5 Mollison P L *Blood transfusi n in clinical medicine* Blackwell Scientific Publications Oxford London Edinburgh and Melbourne 197 5th ed p 59

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Table 4. *Total haemoglobin mass (THb) and the volumes of red cells and plasma of the infants before and after the exchange and of the given and removed blood*

All values are expressed per kg body weight: mean values and S.D. of ten exchange transfusions. The right hand column shows the fluid transferred from the red cells to the plasma

	Infant before *a*	Given *b*	Removed *c*	Infant after *d*	*a+b−c−d*
THb g/kg	12.9±2.0	25.8±2.8	27.4±1.5	11.3±1.6	
Red cell volume ml/kg	40.2±6.2	91.8±9.5	86.9±6.4	35.3±5.8	9.8±3.1
Plasma volume ml/kg	46.1±3.1	78.8±5.6	87.0±5.6	45.6±7.3	

infant gains or loses blood constituents during an exchange transfusion. This question, although seeming simple, could not be answered by a study of the literature, since earlier studies reported only the concentrations of various blood constituents in given and removed blood. In the present report, the volumes of red cells and of plasma and the amounts of haemoglobin gained or lost have been investigated.

The prediction of the infant's RCV from the venous haematocrit was performed according to Bratteby who has pointed out that this should be valid for normal full term infants as well as for prematures (3). The body haematocrit/venous haematocrit ratio of 0.90 is an approximated value but agrees with ratios found in newborns as well as adults (2). Thus the blood volumes could be calculated in the same manner for all infants in the present investigation though the group consisted of both prematures and full term infants.

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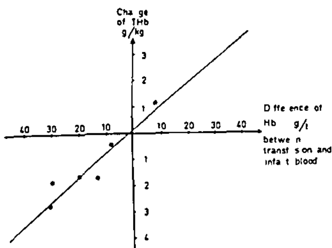


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Table 1 *Concentrations of bilirubin total protein chloride and phosphate of plasma and concentrations of sodium potassium calcium and magnesium of whole blood*

Mean values and S D of ten exchange transfusions

	Conc	Infant before	Given blood	Removed blood	Infant after
P-Bil	μmol/l	225 7±16[illegible] 5[a]	10 4±8 6[a]	143 6±106 0	128 3±90 6
P Protein	g/l	51±8	55±5	53±5	50±8
P-Cl	mmol/l	108 5±4 0[a]	70 8±4 4[a]	101 0±4 0	100 6±4 1
P Phosphate	mmol/l	1 8±0 4[a]	1 8±0 9[a]	2 1±0 4	[illegible] 2±0 4
B Na	mmol/l	78 5±11 9	8[illegible] [illegible]±4 9	84 3±6 9	83 7±6 0
B K	mmol/l	38 5±6 [illegible][a]	33 5±3 1	35 5±3 1	33 7±4 3
B-Ca	mmol/l	1 [illegible]±0 4	1 1±0 [illegible]	1 [illegible]±0 3	1 3±0 3
B Mg	mmol/l	1 9±0 3	1 8±0 [illegible]	1 8±0 [illegible]	1 8±0 3

Significant difference (see text) of infant before/infant after or given blood/removed blood

Bilirubin

Before ET the infants plasma bilirubin concentrations varied between 58 and 482 μmol/l and the plasma bilirubin contents were calculated to vary between 2 57 and 20 01 μmol per kg bodyweight The bilirubin mean concentration of the plasma decreased significantly from 225 7 to 128 3 μmol/l during the ET (Table 1 $p<0\ 025$) On the average the infants plasma pool was calculated to contain 10 21 μmol bilirubin before the ET 0 77 μmol were added and 12 52 μmol removed by the ET (Table 2) After the ET the plasma pool was calculated to contain on average 5 81 μmol bilirubin indicating that on average 7 35 μmol had been formed or transferred to the plasma from other compartments during the ET (Table 2) This increase differed significantly from zero $p<0\ 0025$ Fig 1 shows the correlation between the pre exchange bilirubin concentration and the total amount of bilirubin removed by the ET

Chloride

The mean concentration of plasma chloride decreased significantly from 108 5 to 100 6 mmol/l during the ET (Table 1 $p<0\ 001$) The given amount was 5 60 mmol and the removed amount was 8 81 mmol the difference was significant (Table 2 $p<0\ 001$) The calculated mean amount of plasma chloride of the infants was 5 00 mmol before and 4 58 mmol after the ET indicating an inflow of 2 79 mmol during the

Table 2 *Total amount of bilirubin total protein chloride and phosphate of plasma and sodium potassium calcium and magnesium of whole blood per kg bodyweight*

Mean values and S D of ten exchange transfusions Column *e* shows the net effect of the exchange i e increase (+) or decrease (−) of the substance in plasma or whole blood

	Conc	*a* Infant before	*b* Given blood	*c* Removed blood	*d* Infant after	*e=d+c−(a+b)* increase (+) decrease (−)
P Bil	μmol	10 21±7 00[a]	0 77±0 53[a]	12 52±8 77	5 81±4 19	+7 35±5 60[a]
P Prot	g	33±0 37	4 38±0 67	4 69±0 55	2 [illegible]8±0 45	+0 [illegible]6±0 51
P Cl	mmol	5 00±0 35	5 60±0 56[a]	8 81±0 58	4 58±0 71	+2 79±0 95[a]
P Phosphate	mmol	0 08±0 0[illegible]	0 14±0 04[a]	0 18±0 03	0 10±0 25	+0 06±0 0[illegible][a]
B Na	mmol	6 74±0 80	14 00±0 84	14 59±1 31	6 73±0 98	+0 58±0 99
B K	mmol	3 33±0 6[illegible][a]	5 71±0 64	6 [illegible] ±0 53	2 70±0 43	−0 12±0 63
B-Ca	mmol	0 10±0 03	0 19±0 04	0 20±0 05	0 11±0 0[illegible]	+0 0[illegible]±0 03
B Mg	mmol	0 16±0 03	0 31±0 04	0 30±0 04	0 14±0 03	−0 03±0 04

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Acta Pædiatr Scand 64 310–314 1975

EXCHANGE TRANSFUSIONS WITH CONCENTRATED ACD BLOOD

II *Effects on Bilirubin Total Protein Chloride Phosphate Calcium Magnesium and Potassium*

A KREUGER

From the Department of Paediatrics and the Blood Center University Hospital Uppsala Sweden

ABSTRACT Kreuger A (Department of Paediatrics University Hospital Uppsala Sweden) Exchange transfusions with concentrated ACD blood II Effects on bilirubin total protein chloride phosphate calcium magnesium and potassium Acta Paediatr Scand 64 310 1975 —During ten exchange transfusions with ACD blood (NIH sol B) newborn infants bilirubin plasma protein and electrolyte gain or loss were studied quantitatively The loss of bilirubin was closely correlated to the pre-exchange bilirubin concentration On the average there was a significant loss of plasma chloride of 3 21 mmol per kg bodyweight There were no significant gains or losses of plasma protein or sodium potassium magnesium and calcium of whole blood during the ET There was a calculated significant inflow of whole blood calcium from the extravascular space of 0 02 mmol per kg bodyweight together with a calculated significant increase of plasma phosphate of 0 06 mmol per kg bodyweight

KEY WORDS Exchange transfusions newborn

In a preceding paper (4) red cell measurements of the given and removed blood during ten exchange transfusions (ET) were described In the present investigation of the same patients at the same ETs the total amounts of bilirubin total protein chloride and phosphate in plasma gained or lost were determined as well as the sodium potassium calcium and magnesium content of whole blood

METHODS

Chloride was determined by titration with mercury nitrate sodium potassium and calcium by flame photometry magnesium was determined by atomic adsorption and phosphate according to Gomori (3) The plasma protein concentration was determined by its ultraviolet light absorption (7) Bilirubin was determined according to Michaelsson (5) Determinations on whole blood were made after freezing and thawing of the blood

Calculations

At each exchange transfusion the total amount of a blood constituent given or removed was calculated from the volumes reported in the preceding paper (4) and the concentrations determined in the present investigation For comparison the values are given per kg bodyweight The predicted blood and plasma volumes of the infants before and after the ET (4) were used for the calculations of their total blood and total plasma content of the constituents studied respectively

RESULTS

Table 1 shows the concentrations and Table 2 the total amounts of bilirubin total protein chloride phosphate calcium magnesium potassium and sodium in the children s plasma or whole blood before and after the ET and in the administered and removed blood All total amounts are here given per kg bodyweight of the infant

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Table I shows the concentrations and Table the total amounts of bilirubin total protein chloride phosphate calcium magnesium potassium and sodium in the children s plasma or whole blood before and after the ET and in the administered and removed blood All total amounts are here given per kg bodyweight of the infant

In newborns treated with ET because of hyperbilirubinaemia the post transfusion rise of plasma bilirubin partly depends on the equilibration of bilirubin between the plasma and the extravascular pools However free haemoglobin from non viable red blood cells of the transfusion blood will increase the bilirubin formation The 24 hour post transfusion red cell survival is not known but can be expected to vary between 95 and 99% depending on the age of the transfusion blood (cp Mollison 6) If the fraction of red cells haemolysed during the first day after transfusion is 3% this corresponds to a bilirubin load of 18 μmol/kg i e about the same as the removed amount in some of the children with hyperbilirubinaemia (Fig 1) This means that in a newborn with hyperbilirubinaemia due to immature conjugation but without hyperhaemolysis ET serves more as means of preventing excessive hyperbilirubinaemia until normal bilirubin excretion is established This also indicates the value of using transfusion blood that is as fresh as possible

Chloride

The ACD solution does not contain any chloride and it is hypo osmolar which promotes an inflow of water to the cells This and the removal of ACD plasma mixture before the ET explain the low chloride content of the administered blood The mean loss during the ET was 3 21 mmol which constitutes about 5% of the total chloride mass of the body (1) However the plasma chloride conc was maintained probably by inflow from the extravascular space The mean loss of about 3 mmol per kg bodyweight should be compared with the normal daily intake of chloride from breast milk of about 1 mmol per kg bodyweight at this age (2)

Phosphate

The concentration of plasma phosphate of donor blood increases with the storage time due to the decomposition of intracellular organic phosphates mainly 2 3-diphosphoglycerate However in the ET we use fresh blood and our investigation has shown that there is a small total loss of plasma phosphate of on average 0 04 mmol per kg bodyweight during the ET and a small but significant increase in plasma phosphate of on average 0 06 mmol per kg bodyweight ($p<0\ 001$) This increase in phosphate is difficult to explain Probably it is not due to haemolysis during ET Hypothetically it is more probably an inflow from the extravascular space and a mobilisation together with calcium from the skeleton caused by citrate administration and temporarily decreased pH

Calcium

No additional calcium was given during the ET We have measured the total calcium amount of whole blood There was a small loss of on average 0 03 mmol calcium per kg bodyweight during the ET and this loss was compensated by a significant inflow of calcium from the extravascular space of on average 0 04 mmol per kg bodyweight ($p<0\ 05$) The small loss of total calcium during an ET constitutes about 0 01% of the total body calcium (1) Ionized calcium was not separately studied in this investigation

Sodium

100 ml ACD solution contain 1 32 g sodium citrate enough to raise the sodium concentrations of the administered blood slightly However we could not see any significant difference of concentration of sodium in whole blood nor was there any significant gain or loss of sodium during the ET

Potassium

The plasma potassium conc increases in transfusion blood with the storage time of the blood This increase is due to an outflow of potassium from the red cells and the whole blood potassium concentration remains unchanged during storage In our series the plasma potassium

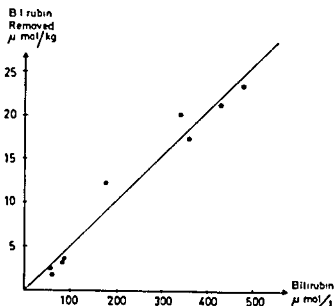


Fig. 1 The correlation between the bilirubin concentration in μmol/l plasma before the ET and the amount in μmol/kg bodyweight of bilirubin removed by the ET $r=0\ 98$

ET (Table 2) The inflow differed significantly from zero ($p<0\ 001$)

Phosphate

There was a significant increase of the plasma phosphate s mean concentration during the ET from 1 8 to 2 2 mmol/l (Table 1 $p<0\ 01$) On the average 0 14 mmol were given and 0 18 mmol were removed forming a net loss of 0 04 mmol during the ET (Table 2) The loss was significant ($p<0\ 0025$) The calculated mean content of plasma phosphate of the infants was 0 08 mmol before and 0 10 mmol after the ET Consequently there was an inflow of plasma phosphate of on average 0 06 mmol during the ET (Table 2) This increase differed significantly from zero ($p<0\ 001$)

Calcium

No additional calcium was given during the ETs No significant changes of plasma or whole blood concentrations were noted during the ETs (Table 1) On the average the removed amount was 0 20 mmol and the given amount 0 19 mmol Thus a net loss of 0 01 mmol was formed (Table 2) The calculated mean content of the infants whole blood was 0 10 mmol before and 0 11 mmol after the ET There was a small inflow of on average 0 02 mmol from the extravascular space during the ET The inflow differed significantly from zero (Table 2 $p<0\ 05$)

Potassium

The mean concentration of potassium in whole blood was 22 5 mmol/l of given blood and 35 5 mmol/l of removed blood (Table 1) The corresponding plasma potassium mean concentration was 5 6 mmol/l of given blood and 4 4 mmol/l of removed blood

The mean concentration of potassium in the infants whole blood decreased significantly from 38 5 to 33 7 mmol/l during the ET (Table 1 $p<0\ 05$) The corresponding calculated whole blood potassium decreased from 3 33 to 2 70 mmol/kg (Table 2 $p<0\ 05$) The total effect of the ET was a small insignificant loss of 0 12 mmol whole blood potassium (Table 2)

Sodium magnesium and total protein

On the average there were no significant changes of the concentrations or total amounts of whole blood sodium whole blood magnesium or plasma protein in the infants during ET Nor were there any significant differences in these parameters between given and removed blood (Tables 1 and 2)

DISCUSSION

Bilirubin

As expected there was a close correlation between the pre exchange plasma bilirubin concentration of the child and the amount of bilirubin removed (Fig 1 $r=0\ 98$) The correlation equation could be expressed as $Y=0\ 051X+0\ 257$ where X=plasma bilirubin concentration before ET in μmol/l and Y=removed amounts of bilirubin in μmol/kg body weight If the pre exchange conc was 350 μmol/l about 18 μmol bilirubin were removed per kg bodyweight Lower pre exchange bilirubin concentrations consequently gave less bilirubin removal during the ET

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HISTOLOGICAL AND BIOCHEMICAL CHANGES IN NEONATAL THYROID TISSUES

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ABSTRACT Etling N and Larroche J C (Unite Inserm 30 Hopital des Enfants Malades Centre de Recherches biologiques neonatales Hopital de Port Royal Paris France) Histological and biochemical changes in neonatal thyroid tissues. Acta Paediatr Scand 64 315 1975 —The thyroid tissues of 17 infants who died between 3 hours and 46 days after birth were studied by histological and biochemical techniques The morphological aspect and the iodine content of these tissues are not related to the gestational age of the neonates but they are related to the survival time There are dramatic events early after birth desquamation of the epithelium and absence of colloid low iodine content of tissue extract (less than 1 μg ^{127}I per mg of protein) and low percentage of thyroglobulin (less than 10 %) 24 hours after birth the vesicles fill with colloid and the epithelium is cuboidal the iodine content of the protein increases (between 1 and 2 μg ^{127}I per mg protein) as well as the thyroglobulin percentage (around 20 %) One week after birth there is a maximum of colloid and flat epithelium the iodine content of the protein extract is much higher (more than 2 μg ^{127}I per mg protein) as is thyroglobulin percentage (up to 40 %) Our studies of thyroid tissues of neonates suggest that a leakage of colloid iodine and thyroglobulin takes place in the perinatal period this phenomenon being followed by their rapid repletion

KEY WORDS Newborn thyroid tissue colloid vesicle thyroglobulin content iodine content gestational age

The fetal thyroid gland appears as a cellular mass arranged in cords separated by vascular mesenchyme Toward the third month of gestation these cords acquire their follicular appearance the central mass of colloid being surrounded by a simple cuboidal or low columnar epithelium this maturation starts from the periphery and progresses toward the central part of the gland During the second and third trimester the pattern of the gland does not vary much

Iodine metabolism has been studied in fetal thyroid glands from abortus either by *in vitro* incubation (18) organ culture (22) or ^{131}I administration to the pregnant mother (26) The fetal thyroid gland begins to accumulate radioiodine by the 10th–12th week of gestation and synthesis of iodothyronine was shown to take place at about the 17th week It is well established that newborn sera present a transient increase of thyrotropin (TSH) in the early minutes of postnatal life (4 8) and that the triiodothyronine (T_3) and thyroxine (T_4) contents present also an increase during the early hours of life (1)

If there is no controversy on the embryologic development of the thyroid gland and the appearance of the colloid as well as the dramatic changes of serum TSH and hormones content that surround birth there is no biochemical information on the thyroid tissue within the first days of life Our purpose is to review the morphological features and biochemical correlations in neonatal thyroid tissues and to relate the changes in the tissues to the known blood modifications

concentrations were only moderately increased and no cardiac arrhythmias were noticed during the ETs

The whole blood potassium content of the infants decreased significantly during the ETs from a mean of 3 33 to 2 70 mmol per kg bodyweight (Table 2 $p<0$ 05) This might be explained by a significantly higher whole blood potassium conc in the removed than of the given blood (Table 2 $p<0$ 0025) It is difficult to understand completely the change of potassium distribution during the ET One explanation might be the high glucose concentration of the ACD blood promoting an inflow of potassium into cells although the figures in our investigation poorly reflect the complexity of the glucose insulin effect on potassium and cells

Magnesium

Most of the magnesium of the blood is found intracellularly We have measured the total amount of Mg in samples of whole blood There were no significant changes of whole blood magnesium during the ETs However ionized magnesium was not separately studied in this investigation

REFERENCES

1 Diem K *Wissenschaftliche Tabellen* J R Geig A G Basel 1960 6th ed p 486
2 Fomon S J *Infant nutrition* W B Saunders Philadelphia and London 1967 p 199
3 Gomori G A modification of the colorimetr phosphorus determination for use with the photo electric colorimeter *J Lab Clin Med* 27 955 1941-4
4 Kreuger A & Wranne L Exchange transfusions with concentrated ACD-blood I Effects on the infant s re cell volume plasma volume and haemoglobin mass *Acta Paediatr Scand* 64 305 1975
5 Michaelsson M Bilirubin determination in serum and urine *Scand J Clin Lab Invest* Suppl 56 1961
6 Mollison P L *Blood transfusion in clinical medicine* Blackwell Scientific Publications Oxford London Edinburgh and Melbourne 1972 5th ed p 12
7 Patrick R L & Thiers R E The direct spectro photometric determination of protein in cerebrospina fluid *Clin Chem* 9 283 1963

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HISTOLOGICAL AND BIOCHEMICAL CHANGES IN NEONATAL THYROID TISSUES

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ABSTRACT Etling N and Larroche J C (Unite Inserm 30 Hopital des Enfants Malades Centre de Recherches biologiques neonatales Hopital de Port Royal Paris France) Histological and biochemical changes in neonatal thyroid tissues Acta Paediatr Scand 64 315 1975 —The thyroid tissues of 17 infants who died between 3 hours and 46 days after birth were studied by histological and biochemical techniques The morphological aspect and the iodine content of these tissues are not related to the gestational age of the neonates but they are related to the survival time There are dramatic events early after birth desquamation of the epithelium and absence of colloid low iodine content of tissue extract (less than 1 μg ^{127}I per mg of protein) and low percentage of thyroglobulin (less than 10%) 24 hours after birth the vesicles fill with colloid and the epithelium is cuboidal the iodine content of the protein increases (between 1 and 2 μg ^{127}I per mg protein) as well as the thyroglobulin percentage (around 20%) One week after birth there is a maximum of colloid and flat epithelium the iodine content of the protein extract is much higher (more than 2 μg ^{127}I per mg protein) as is thyroglobulin percentage (up to 40%) Our studies of thyroid tissues of neonates suggest that a leakage of colloid iodine and thyroglobulin takes place in the perinatal period this phenomenon being followed by their rapid repletion

KEY WORDS Newborn thyroid tissue colloid vesicle thyroglobulin content iodine content gestational age

The fetal thyroid gland appears as a cellular mass arranged in cords separated by vascular mesenchyme Toward the third month of gestation these cords acquire their follicular appearance the central mass of colloid being surrounded by a simple cuboidal or low columnar epithelium this maturation starts from the periphery and progresses toward the central part of the gland During the second and third trimester the pattern of the gland does not vary much

Iodine metabolism has been studied in fetal thyroid glands from abortus either by *in vitro* incubation (18) organ culture (22) or ^{131}I administration to the pregnant mother (26) The fetal thyroid gland begins to accumulate radioiodine by the 10th–12th week of gestation and synthesis of iodothyronine was shown to take place at about the 17th week It is well established that newborn sera present a transient increase of thyrotropin (TSH) in the early minutes of postnatal life (4 8) and that the triiodothyronine (T_3) and thyroxine (T_4) contents present also an increase during the early hours of life (1)

If there is no controversy on the embryologic development of the thyroid gland and the appearance of the colloid as well as the dramatic changes of serum TSH and hormones content that surround birth there is no biochemical information on the thyroid tissue within the *first days of life* Our purpose is to review the morphological features and biochemical correlations in neonatal thyroid tissues and to relate the changes in the tissues to the known blood modifications

concentrations were only moderately in creased and no cardiac arrhythmias were noticed during the ETs

The whole blood potassium content of the infants decreased significantly during the ETs from a mean of 3 33 to 2 70 mmol per kg body weight (Table 2 $p<0\ 05$) This might be explained by a significantly higher whole blood potassium conc in the removed than of the given blood (Table 2 $p<0\ 0025$) It is difficult to understand completely the change of potassium distribution during the ET One explanation might be the high glucose concentration of the ACD blood promoting an inflow of potassium into cells although the figures in our investigation poorly reflect the complexity of the glucose insulin effect on potassium and cells

Magnesium

Most of the magnesium of the blood is found intracellularly We have measured the total amount of Mg in samples of whole blood There were no significant changes of whole blood magnesium during the ETs However ionized magnesium was not separately studied in this investigation

REFERENCES

1 Diem K *Wissenschaftliche Tabellen* J R Geigy A G Basel 1960 6th ed p 486
2 Fomon S Y *Infant nutrition* W B Saunders Philadelphia and London 1967 p 199
3 Gomori G A modification of the colorimetric phosphorus determination for use with the photoelectric colorimeter *J Lab Clin Med 27* 955 1941–42
4 Kreuger A & Wranne L Exchange transfusions with concentrated ACD-blood I Effects on the infant s red cell volume plasma volume and haemoglobin mass *Acta Paediatr Scand 64* 305 1975
5 Michaelsson M Bilirubin determination in serum and urine *Scand J Clin Lab Invest* Suppl 56 1961
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Table 1 *Neonatal clinical data*
HMD=Hyaline Membrane Disease

Subject	Sex	Body weight (g)	Gest age (weeks)	Age at death	Weight thyroid (mg)	Presumed cause of death	Histology colloid content
MON no 1	M	2 180	35	3h10	4 000	Obstetrical trauma	0
SCH no 2	M	2 000	31	9h35	1 346	Hemolytic disease of new born	0
IZZ no 3	M	1 700	32	11h30	650	HMD	0
ST L no 4	F	1 860	32	16h20	1 220 6	HMD	+++
BER no 5	F	1 430	29	17h	788 8	HMD	0
DEL no 6	F	1 100	29	26h	450 7	HMD	++
BIL no 7	M	1 910	37	31h	809 6	HMD	++
COU no 8	M	1 180	30	32h	700	Pulmonary hemorrhage	+++
ROT no 9	M	2 300	37	34h	679 5	HMD	+++
JEA no 10	M	1 300	31	35h	543 5	Hemolytic disease of new born	+
MAX no 11	M	1 660	30/31	36h	987	Second twin HMD	+
HER no 12	F	1 180	30/31	38h	570 7	Congenital heart disease	++
ETI no 13	M	1 130	29	2d	420	HMD	++
BOR no 14	M	1 240	29	3d	490	HMD	++
TEP no 15	F	975	26	17d	474	Prematurity intraventricular hemorrhage	++++
LAN no 16	M	3 650	36	13d	946 5	Diabetic mother hepatic necroses	++++
ALE no 17	M	2 005	33	49d	1 152	Respiratory distress syndrome	++++

MATERIAL

Seventeen thyroid glands of premature and full term newborns were obtained from autopsies performed within 10 hours (mean) after death specimens are kept at 4 C before dissection The gland is removed and weighed Half is immediately stored in ice and rapidly frozen for biochemical studies the other half is processed in paraffin for histological examination Body weight gestational age length of life sex thyroid weight and presumed cause of death are shown in Table 1

METHODS

The lobe used for microscopy is fixed either in formalin or in Bouin s solution and sections are stained by hematoxylin eosin and/or Masson s trichrome

The other lobe is kept at −20 and three extractions are performed with 0 14 M NaCl (700 mg wet tissue per ml) The extracts are centrifuged in a refrigerated MSE centrifuge at 8 000 g Stable iodine (^{127}I) is measured in the pellet solubilised into N NaOH and in the supernatant or soluble proteins of the extract ^{127}I is determined by a semi automatic micromethod with the Technicon Autoanalyser (12) Each sample is mineralised with sulfonitroperchloric mixture and the catalytic effect of iodine on the reduction of ceric ions by arsenous acid is used using this method amount of 1 to 20 ng can be determined with a 5% precision Protein contents are estimated by UV readings at 260 and 280 nm (25)

The proteins of the extract are separated by 5% polyacrylamide gel electrophoresis according to the technique of Barka (3) in a PleugerAcrylophor apparatus at room temperature 3 mA per gel during about 1 hour in a 0 02 M Tris glycine buffer at pH 8 5 The samples are stained with amido black 0 5% in 2% acetic acid and destained with acetic acid until complete decoloration of the background The optical densities of the stained proteins are recorded with a Gilford Spectrophotometer at 610 mm with a 10% precision The results are compared with a reference thyroglobulin lyophilised The thyroglobulin and other protein contents are measured by planimetry and/or weighing the peak surfaces and percentages are calculated

RESULTS

(A) *Histological Examination*

Morphology varies greatly and can be classified into 2 main types

Type I thyroids free of colloid with desquamation of the epithelium and desintegration of follicules (Fig 1)

Type II thyroids containing colloid This type is classified into subgroups according to the number of colloid filled vesicles their size and the appearance of the epithelium

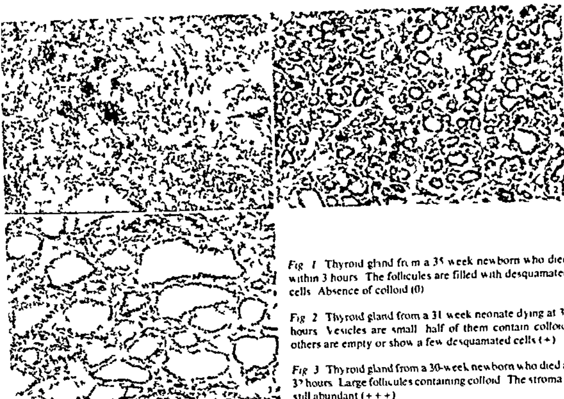


Fig 1 Thyroid gland from a 35 week newborn who died within 3 hours The follicules are filled with desquamated cells Absence of colloid (0)

Fig 2 Thyroid gland from a 31 week neonate dying at 36 hours Vesicles are small half of them contain colloid others are empty or show a few desquamated cells (+)

Fig 3 Thyroid gland from a 30-week newborn who died at 37 hours Large follicules containing colloid The stroma is still abundant (+ + +)

Subgroup + few vesicles containing colloid at the periphery The rest of the gland appears as a rather compact organ made up of many vesicles of varying size well recognizable but without true lumen and without colloid The epithelium is cuboidal high however there is very little or no desquamation at all (Fig 2)

Subgroup ++ increased number of colloid filled vesicles mostly in the periphery Few empty vesicles in the centre with still some solid buds

Subgroup +++ all vesicles are distended with colloid the epithelium is still cuboidal (Fig 3)

Subgroup ++++ represents the maximum colloid content we have observed rather uniformly distributed with a flat epithelium (adult like feature)

These morphological variations were related neither to birth weight or gestational age nor to sex By contrast they seem to be related to length of life

Of these 17 infants 5 died within 24 hours 4 of them had neither normal vesicles nor colloid the epithelium was vacuolated and desquamated into the lumen However one (ST L no 4) had mature vesicles filled with normal looking colloid 12 infants survived more than 24 hours They all exhibited colloid in the vesicles ranging from + to ++++

We observed that from 15 hours to 6 days the vesicles open up from the periphery to the centre and progressively fill up with colloid The epithelium still rather high is never vacuolated or detached from the basal membrane it will flatten with survival

From 7 days on the vesicles enlarge and fill with colloid the epithelium flattens and resembles the adult type

Because of the rather small number of cases included in this parallel study of morphology and biochemistry we surveyed the thyroid gland of 133 other cases from the files of the department of pathology Table 2 shows the

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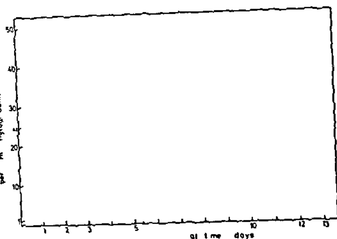


Fig 5 Relation between thyroglobulin percentage in total proteins and survival time (days) in thyroid tissue extracts of neonates

Fig 5 shows the relation between the percentage of thyroglobulin in total extractable proteins and the survival time of the neonates A few hours after birth the thyroglobulin percentage is low (around 10 for MON and SCH) but beyond 12 hours the amount increased to 20% or more (except BER only 14) Only two neonates reached a level of more than 30% thyroglobulin (ROT 35 HER 38 5) before 3 days of age and the thyroglobulin percentage of neonate LAN is more than 40 slightly less than that of human adult extracts (50–70)

DISCUSSION

These different histological features are already described in the literature Desquamation of the epithelium and absence of colloid are diversely interpreted For Gloor (9) and Murray (15) these aspects represent a post mortem autolysis whereas Norris (17) Krinskaja (11) and Nicod (16) have no interpretation of the phenomena Allara (2) did not notice this difference between intra uterine life birth and long survival Hesselberg as early as 1910 (10) reivewed the already abundant descriptive literature on the thyroid and considered that the desquamation and the disappearance of colloid were due to damage during birth by reason of the relative narrowness of the canal More recently Sclare (21) Muller & Ramsch (14) and Sagreiya & Emery (20) interpreted these changes as a manifestation of hyperactivity

We have eliminated every case of post mortem autolysis (long delay between death and autopsy) and we consider that the desquamation of the vesicular epithelium is not an artefact Rarely described from 48 hours to 6 days and never beyond this period this feature is an other argument against the role of autolysis

We know nothing about the tissue itself during the first 5 days of life The changes we observed here occur very early after birth It was impossible to verify our results using ^{131}I To be able to understand the probably transient depletion of iodine it was necessary to make stable iodine determinations with a microtechnique such methods were not previously available The acrylamide gel electrophoresis is a recent useful method because of its great sensitivity and resolution Two years ago however Slebodzinski (23) by giving ^{131}I Na to pregnant sheep 3 to 8 days before parturition demonstrated in their lambs within 15 min of birth a rapid outflow of thyroidal radioiodine and a decrease in iodine content (seen by

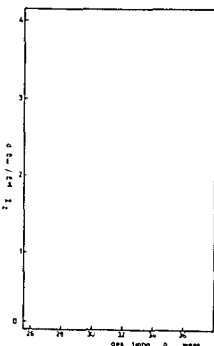


Fig 4 a Relation between I/P (μg [127]I per mg protein) and gestational age (weeks) in thyroid tissue extracts of neonates

distribution of colloid free containing vesicles The morphological features described in this retrospective series of 133 cases are similar to those described in the series under study (17 cases) and the percentage of colloid free vesicles is about the same in both series (62 8% to 80% within 24 hours of life and 0 to 5 1% beyond 24 hours of life)

(B) *Biochemical Analysis*

Iodine content

The relation between the iodine content of a mg of protein of thyroid extract (μg [127]I/mg protein or I/P) and the gestational age of the neonates is shown in Fig 4*a* There is no correlation between these two parameters the iodine content of neonatal thyroid tissue remains independent of its maturation

The correlation between the I/P ratio and the survival time of neonates is shown in Fig 4*b* The neonates with a survival time shorter than 24 hours show an I/P ratio lower than 1 (except ST L) among them those with a survival time below 10 hours (MON SCH) show an I/P ratio lower than 0 5 Among the thyroid extracts from the 9 neonates with a survival time

Table 2 *Distribution of colloid free vesicles*

	Age at death	No of cases	No colloid	Absence of colloid (% cases)
(1)	<24 h	35	22	62 8
(2)	24–48 h	33	3	9
(3)	49 h – 6 days	38	2	5 2
(4)	>6 days	27	0	0

The differences between 1 and 2 1 and 3 1 and 4 are highly significant $p<0\,001$

between 26 and 72 hours the I/P of each extract is higher than 1 (except MAX 0 5 and BOR 0 89) and lower than 2 (except ROT 2 32) The last 3 thyroid extracts corresponding to a post natal life longer than a week show an I/P ratio above 2 The iodine content of thyroidal proteins from neonatal tissue extracts seems to be related to survival time The iodine content of one mg of protein of a thyroid extract from a normal adult tissue is 3 5–4 μg slightly higher than in neonates living more than a week

Thyroglobulin content

The percentage of thyroglobulin protein extracted from the colloid of neonatal tissue (see Methods) varies according to the survival time

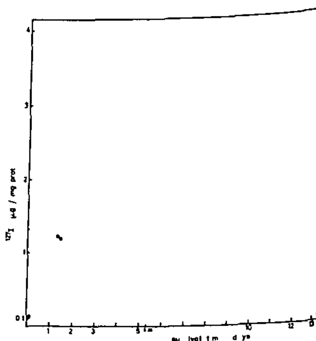


Fig 4 b Relation between I/P (see Fig 4*a*) and survival time (days) in thyroid tissue extracts of neonates

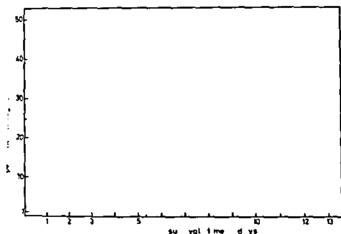


Fig 5 Relation between thyroglobulin percentage in total proteins and survival time (days) in thyroid tissue extracts of neonates

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staining the tissue) he related the two events

Our studies have shown that within the first hours of life the very low colloid content and the epithelium desquamation are closely related to the low iodine and thyroglobulin contents One case deserves comment ST L (case 4) shows many colloid filled vesicles a high I/P ratio and a large percentage of thyroglobulin in spite of his short survival this cannot be explained on a physiological or biochemical basis but we know that about 30% of newborns may exhibit colloid of filled vesicules within 24 hours of life

After 24 hours we demonstrate a progressive filling of the thyroid gland on a morphological as well as on a biochemical basis In this group BOR (case 14) represents the lower limit this may be due to his young gestational age (29 weeks) ROT (case 9) represents the upper limit which may be due to his relative maturation (37 weeks)

After 12 days of life the histological aspect and iodine content of the tissue and percentage of thyroglobulin tend to reach adult values

The very low amount of iodine and thyroglobulin observed in the perinatal period suggests a probable leakage of the iodo compounds at an unknown moment at/or about the time of birth followed by a regular and rapid repletion of iodine and thyroglobulin a few hours after birth One week after birth the tissue trapping iodine and protein material is approaching the adult proportions

The iodine content changes observed in the thyroid tissues during the neonatal period demonstrate a thyroid dysfunction independent of the gestational age These data are supported by the abrupt TSH increase (4 8) the changes of serum T_3 and T_4 concentrations (1 6) and the higher level of blood thyroglobulin in the same period (24) The leakage of colloid and thyroglobulin may explain the PBI increase and its plateau 24–48 hours after birth described in full term newborns by Danowski (5) and in prematures by Marks & Man (13) and by Perry et al (19)

We have established through histologic and biochemical investigation that the level of iodine colloid and thyroglobulin reached their lower level within few hours of birth this phenomenon being followed by their rapid repletion Work on thyroglobulin composition will be the subject of further publication (7)

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REFERENCES

1 Abuid J Stinson A & Larsen P R Serum triiodothyronine and thyroxine in the neonate and the acute increases in these hormones following delivery *J Clin Invest* 52 1195 1973

2 Allara E Sulla structuura della ghiandola tiroide del neonato *Arch Ital Anat Embriol* 56 273 1951

3 Barka T Studies of acid phosphatase I electrophoretic separation of acid phosphatases of rat liver on polyacrylamide gels *J Histochem Cytochem* 9 542 1961

4 Czernichow P Greenberg A H Tyson J & Blizzard R M Thyroid function studied in paired maternal-cord sera and sequential observations of thyrotropic hormone release during the first 72 hours of life *Pediatr Res* 5 53 1971

5 Danowski T S Johnston S Y Price W C McKelvy M Stevenson S S & McCluskey E R Protein bound iodine in infants from birth to one year of age *Pediatrics* 7 240 1951

6 Erenberg A Phelps D L Lam R & Fisher D A Total and free thyroid hormone concentrations in the neonatal period *Pediatrics* 53 211 1974

7 Etling N Iodination of human neonates thyroglobulin (manuscript in preparation)

8 Fisher D A & Odell W D Acute release of thyrotropin in the newborn *J Clin Invest* 48 1670 1969

9 Gloor H U Beitrag zur Kenntnis der Schilddruse des Neugeborenen *Frankf Z Pathol* 34 504 1926

10 Hesselberg C Die menschliche Schilddruse der foetalen Periode und den ersten 6 Lebensmonaten *Frankf Z Pathol* 5 322 1910

11 Krinskaja V Zur frage der Struktur der Neugeborenen schilddruse *Frankf Z Pathol* 43 41 1932

12 Lachiver F & Boulu Lapointe F Micro-dosage semi automatique de l iode To be published

13 Marks A M & Man E M Serum butanol extractable iodine concentration in prematures *Pediatrics* 35 753 1965

14 Muller E & Ramsch R Schilddrusenbefunde bei Tot und Neugeborenen *Frankf Z Pathol* 75 425 1966

15 Murray I The thyroid gland in the full term human fetus and in the newly born infant *Br Med J 1* 5 1927
16 Nicod J L La thyroïde dans la période périnatale *Schweiz Med Wochenschr 91* 6 6 1961
17 Norris E M The morphologenesis of the follicules in the human thyroid gland *Am J Anat 20* 411 1916
18 Olin P Ekholm R & Almqvist S Biosynthesis of thyroglobulin related to the ultrastructure of the human fetal thyroid gland *Endocrinology 87* 1000 1970
19 Perry R E Hodgman J E & Starr P Maternal cord and serial venous blood protein bound iodine thyroid binding globulin thyroid binding albumin and prealbumin values in premature infants *Pediatrics 35* 759 1965
20 Sagreiya K & Emery J L Perinatal thyroid discharge A histological study of 1 225 infants thyroids *Arch Dis Child 45* 746 1970
21 Sclare G The histological structure of the thyroid in the newborn *Scott Med J 1* 251 1956
22 Shepard T H Onset of function in the human fetal thyroid biochemical and radioautographic studies from organ culture *J Clin Endocrinol Metab 27* 945 1967
23 Slebodzinski A Acute depletion on the hormonal iodine stores from the thyroid gland after birth in lambs *J Endocrinol 53* 195 1972
24 Van Herle A J Uller R P Matthews N L & Brown J Radioimmunoassay for measurement of thyroglobulin in human serum *J Clin Invest 52* 13 0 1973
25 Warburg O & Christian W Isolierung und Kristallisation des Gärungsferments enolase *Biochem Z 310* 384 1941
26 Yamazaki F Noguchi A & Slingerland D W The development of hormonal biosynthesis in human fetal thyroids *J Clin Endocrinol Metab 19* 1437 1959

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MORTALITY AND LIFE-TABLE IN DOWN S SYNDROME

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ABSTRACT Øster J Mikkelsen M and Nielsen A (Department of Paediatrics Randers Centralsygehus Randers John F Kennedy Institute Glostrup and the Institute for Human Genetics University of Copenhagen Denmark) Mortality and life-table in Down s syndrome Acta Paediatr Scand 64 322 1975 —The causes of death in 130 patients with Down s Syndrome and mortality rates from a material of 524 patients were tabulated a life-table for the ages over 5 years was constructed An overall death rate of 5–7 times the general population rate was found No sex difference was observed The excess mortality was especially high for heart disease and respiratory disease Also infectious diseases others than pneumonia and tuberculosis showed high mortality rates

KEY WORDS Down s Syndrome mortality life table infections malignancies

The first life table for persons with Down s Syndrome (D S) was constructed in 1955 by Record & Smith (12) Since then a number of life tables have been compiled especially for age groups below 10 years (1 2 5 6 4) In 1964 Øster et al (17) reported on the mortality and causes of death of persons with D S previously examined by Øster (16) A higher mortality rate was found than in the general population but at that time data were not sufficient to construct a life table especially not for age groups over 45 years Since then data have accumulated and we can now present mortality data and a life table for persons with D S including older individuals

MATERIAL

The original material consisted of all living persons with D S in 1949 (526 cases) from a well-defined area of Denmark who were examined clinically by Øster (16) Two were subsequently excluded because the diagnosis was incorrect (17) All cases were traced in 1960 and those surviving again in 1972 For the decreased death certificates were checked and if pertinent hospital records examined Often when multiple causes of death were listed e g pneumonia and congenital heart disease then the direct cause of death pneumonia was selected It was noted if the person lived at home or in an institution as well as the period of institutionalisation

RESULTS

Of the 524 cases 73 had died before January 1 1960 (17) a further 57 died before January 1 1972 At that date 394 persons were alive and 130 had died

The number of deaths for males and females is compared separately for the two periods the total number of deaths is compared The ratio between observed and population mortality for patients older than 10 years went down from 7 2 to 5 5 The decrease was lower for females than for males (Table 1) The differences however are non significant In Table 2 the material is divided into persons living at home or in institutions A slight non significant tendency was found towards higher ratios in institutions this was reduced during the period 1960–71

The mortality rates and causes of death of the 130 individuals are given in Table 3 They are

Table 1 *Number of deaths compared with mortality in population corrected for age*

	Period					
	1949–59			1960–71		
Age	Obs	Cal	Obs / Calc	Obs	Calc	Obs / Calc
Males						
0– 4	7	0 [illegible]	3	–	–	–
5– 9	5	0 [illegible]4	[illegible]1	–	–	–
10–14	3	0 0	15	1	0 06	17
15–44	1	1 67	7	19	[illegible] 6[illegible]	7
45–	11	1 88	6	15	3 76	4
Over 10	6	3 75	7 0	35	6 44	5 4
Females						
0– 4	10	0 11	91	–	–	–
5– 9	8	0 11	73	–	–	–
10–14	–	0 11	–	–	0 03	–
15–44	8	1 36	6	8	1 75	5
45–	9	0 78	1[illegible]	14	[illegible] 17	6
Over 10	17	[illegible] 25	7 6	[illegible]	3 94	5 6
Both Sexes						
Total	73	6 68	10 9	57	10 39	5 5
Over 10	43	6 00	7 [illegible]	57	10 39	5 5

compared for the period 1949–59 with the general mortality of Denmark in 1955 and for the period 1960–71 with the mortality in 1965

The two main mortality causes were respiratory diseases mostly pneumonia and heart disease These two large groups were compared for four age groups (Tables 4 and 5)

The mortality from respiratory diseases was very high especially for the age group 5 to 14 years Heart disease showed a very high mortality rate especially for the age group 0–14 years Infectious diseases (others than pneumonia) and senile disease including apoplexia showed high mortality rates Tuberculosis and suicide were not reported (Table 3)

Malignant conditions included two cases of childhood leukemia Two cases of pancreatic cancer were found The other causes of death from malignancy were cancer of the rectum stomach gallbladder ovary and one case of seminoma

From the data available a sex specific life table was constructed (Table 6) As the data for the age group 0 to 5 years were not sufficient for calculations these years were excluded in the table The probability for survival was calculated by the standard actuary formula

Sex specific survival curves for Danish persons with D S for the general Danish population and a curve for both sexes for Swedish individuals with D S are presented in Figure 1 The Swedish survival curve has been constructed from the data collected by Forssman & Åkesson (7) Here too the age group 0 to 5 years is excluded Even if the curve for the females is somewhat irregular most likely because of the limited number the curve for both sexes is very much the same For the whole period a higher mortality of about six times the normal population rate is observed in the Danish D S population The mortality rate for the age group 40–49 was lower than in the Swedish material

DISCUSSION

Previous studies of mortality rates of adults with D S have all shown a high mortality rate (2 17 5 6 7 3)

Table 2 *Number of deaths compared with mortality in population corrected for age and sex*

	Period					
	1949–59			1960–71		
Age	Obs	Calc	Obs / Calc	Obs	Calc	Obs / Calc
Died at home						
0– 4	8	0 [illegible]0	40	–	–	–
5– 9	4	0 19	[illegible]1	–	–	–
10–14	[illegible]	0 15	13	–	0 04	–
15–44	8	1 45	6	1[illegible]	1 8[illegible]	7
45–	5	0 97	5	6	1 75	3
Over 10	15	57	5 8	18	3 61	5 0
Died in institutions						
0– 4	9	0 13	69	–	–	–
5– 9	9	0 16	56	–	–	–
10–14	1	0 16	6	1	0 05	20
15–44	1[illegible]	1 58	8	15	2 55	6
45–	15	1 69	9	23	4 18	6
Over 10	[illegible]8	3 43	8 [illegible]	39	6 78	5 8

Table 3 *Number of deaths compared with mortality in population corrected for age and sex*

Cause of death	Period 1949–59 Obs	Calc	Obs /Calc	1960–71 Obs	Calc	Obs /Calc
Infectious diseases excl T B and Pneumonia	5	0 097	52	1	0 086	12
Tuberculosis	–	0 128	–	–	0 050	–
Malignant conditions	4	1 180	2	5	3 700	1
Accidents poisoning and violence	2	1 136	2	2	1 471	1
Suicide	–	0 685	–	–	0 914	–
Respiratory diseases	37	0 299	124	22	0 353	62
Cardiac diseases	13	1 213	11	17	3 037	6
Senile diseases and apoplexy	3	0 363	8	4	0 771	5
Diseases of the digestive system / Diseases of the genito-urinary system / Other causes	9	2 640	3	6	0 444 / 0 303 / 1 276	3

Two cases of leukemia

The data collected in this study confirm the findings of a high mortality for all age groups But even if the mortality rates are higher than in the general population many persons with D S reach ages over 50 years This fact is important in counselling parents The higher mortality of Swedes with D S in the older age groups (7) compared with this Danish survey may reflect the more recent follow up of our material Advances with more active therapy in cases of infections and other intercurrent diseases may play a role

The difference between the mortality rates observed in 1964 and in the present study may also in part depend on better treatment but the fact that the individuals are older than in the first observation period may be the more important factor

Deaton found a higher mortality rate in a recent study (3) than observed by us His population was drawn from nine different institutions in Texas In our material a tendency to increased mortality in institutions was observed also this tendency could reflect that institutionalized persons with D S are either more severely handicapped than those living at home or it may reflect a higher number of infections occurring at least in larger institutions It was clearly shown that many more patients were HAA positive in large institutions than in small institutions or at home (14 8 15 13) With the greater susceptibility to infectious

Table 4 *Respiratory diseases Number of deaths compared with mortality in population corrected for age and sex*

Age	Period 1949–59 Obs	Calc	Obs / Calc	1960–71 Obs	Calc	Obs / Calc
0– 4	11	0 153	72	–	–	–
5–14	8	0 016	500	–	0 002	–
15–44	8	0 048	167	11	0 067	164
45–	10	0 082	122	11	0 284	39

Table 5 *Cardiac diseases Number of deaths compared with mortality in population corrected for age and sex*

Age	Period 1949–59 Obs	Calc	Obs / Calc	1960–71 Obs	Calc	Obs / Calc
0– 4	2	0 004	500	–	–	–
5–14	3	0 007	428	1	–	High
15–44	5	0 205	24	7	0 384	18
45–	3	0 997	3	9	2 653	3

able 6 *Life table for persons with Down s ndrome*

ge)	No of year at risk	No of death	Force of mortality (μ)	Cumulative survival rate to start of interval
lales				
)– 4	149	7	0470	
5– 9	443	5	0113	1 00
0–14	625	4	0064	0 95
5–19	756	6	0079	0 9
0– 4	790	8	0101	0 88
5–?9	604	4	0066	0 84
0–34	51?	4	0078	0 81
5–39	381	4	0105	0 78
0–44	257	5	0195	0 74
5–49	??9	5	0?18	0 67
0–54	155	?	01?9	0 60
5–59	103	6	0583	0 56
0–64	55	7	1?73	0 4?
5–69	16	4	?500	0 ??
0–74	7	?	?857	0 064
5–79	1	–	–	0 015
Females				
0– 4	106	10	0943	
5– 9	3??	8	0 48	1 00
10–14	486	–	–	0 88
15–19	637	?	0031	0 88
?0–?4	741	4	0054	0 87
25–?9	660	5	0076	0 85
30–34	53?	?	0038	0 81
35–39	455	1	00??	0 80
40–44	369	?	0054	0 79
45–49	?54	8	0315	0 77
50–54	167	5	0?99	0 66
55–59	77	4	0519	0 57
60–64	30	4	1333	0 44
65–69	11	?	1818	0 ??
70–74	–	–	–	0 090

diseases the higher number of infections in large institutions might lead to a higher mortality rate

The mortality from heart disease and respiratory disease is especially high for the age group below 15 After the age of 15 many of the children with congenital heart defects have died as is also shown by Fabia & Drolette (4) who constructed life tables for children with and without heart defects but the sex difference found by Fabia & Drolette was not observed in the present material The causes of death in this study are in good agreement with the findings of Deaton (3) especially when cardio-respiratory diseases are taken together

As mentioned by Deaton a different interpretation of data is apparently the cause of the different percentages when the two groups are divided

It has been claimed that malignant disease is more common in individuals with D S than in the general population (9) Cancer of the testis was also found in other series (10) as well as one case of seminoma in our series A real association between D S and this neoplasm might exist

The two cases of childhood leukemia are significantly more than expected Persons with D S are more susceptible to infections and as recent studies have indicated virus infection as a cause of leukemia the greater incidence of leukemia in D S children might be a simple consequence of this fact After the age of 10

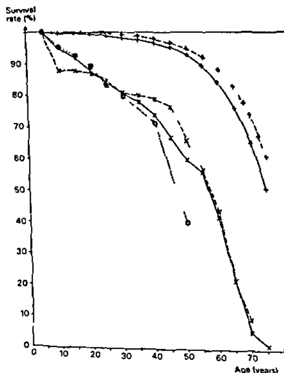


Fig 1 Cumulative survival rates for persons with Down s syndrome alive at 5 years of age compared with cumulative survival rates for the Danish population 1956–60 ×—×/×– – –× Males/females 524 Danish persons with Down s syndrome 1949–71 +—+/+– – –+ Males/females Danish population 1956–60 O O Both sexes Swedish persons with Down s syndrome 1955–67

Table 3 *Number of deaths compared with mortality in population corrected for age and sex*

Cause of death	Period 1949–59 Obs	Calc	Obs /Calc	1960–71 Obs	Calc	Obs /Calc
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Diseases of the digestive system					0 444	
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Other causes					1 226	

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ABNORMAL PROTEOLYSIS IN SICK NEWBORNS

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ABSTRACT Henriksson P and Ekelund H (Department of Paediatrics University of Lund Allmanna Sjukhuset Malmo Sweden) Abnormal proteolysis in sick newborns Acta Paediatr Scand 64 327 1975.—87 newborn infants were studied on their first day of life for defects in the coagulation and fibrinolytic systems The infants were divided into two diagnostic groups one with IRDS the other with mixed neonatal disorders Factor V fibrinogen and fibrin/fibrinogen degradation products (FDP) were abnormal more often than any of the other factors examined The presence or absence of multiple defects appeared to depend on the severity of the illness and its ultimate course Thus 28% of the surviving infants or 85% of those who died had multiple defects The pattern of abnormalities did not differ between the infants with IRDS and those with mixed disorders The multiple defects are ascribed to the following mechanisms (1) impaired synthesis due to vitamin K deficiency and/or liver damage (2) abnormal proteolytic activity stimulated by tissue damage and causing (*a*) an activation of the coagulation process (*b*) activation of the fibrinolytic system or (*c*) of both the coagulation and the fibrinolytic systems Differentiation between these pathways to defective haemostasis are important when deciding upon therapeutic measures in addition to the basic treatment

KEY WORDS Abnormal proteolysis coagulation fibrinolysis IRDS newborn

Changes in the haemostatic mechanism in the pathogenesis of various disorders in newborns have received much space in the literature in the last few years (1 2 8–12 15 25 26 29 40 42 46 47 48 54 61 63) Interest has been focused mainly on the possible role played by disseminated intravascular coagulation (DIC) The contributions to the debate have recently been reviewed by McMillan et al (49) Oski & Naiman (55) and Karpatkin (33)

This paper reports a comparison between a group of newborns with IRDS and a group of infants with mixed neonatal disorders for possible changes in haemostasis

CLINICAL MATERIAL

The material is summarised in Fig 1 where all the 87 infants 33 with IRDS and 54 with other neonatal d s orders are grouped according to gestational age and diagnosis The diagnosis of IRDS was based on Hutchison s criteria (30) though chest x ray was not always possible

The mixed group consisted of infants with unspecified respiratory symptoms not consistent with IRDS apnoea due to immaturity (RIS) postnatal asphyxia and haemolytic disease due to Rhesus immunisation I infants in each group died in the neonatal period 10 infants 6 in the IRDS group and 4 in the mixed group showed evidence of significant intracranial haemorrhage (ICH) One infant in each group survived In these 7 infants the diagnosis of ICH was based on cytologic findings in the cerebrospinal fluid

All the infants received vitamin K 1 mg i m at birth and they were all delivered at the same obstetrical

years no general increase in malignant disease was observed Five of the seven cases of cancer were malignancies of the gastrointestinal tract which does not differ from the general population Cancer of the breast cervix and uterus which are common malignancies in females were not observed in our material This may be an insignificant finding or may reflect a real difference between the general female population and females with D S

REFERENCES

1 Carter C O A life table for mongols with the causes of death *J Ment Defic Res 2* 64 1958
2 Collmann R D & Stoller A A life table for mongols in Victoria Australia *J Ment Defic Res 7* 53 1963
3 Deaton J G The mortality rate and causes of death among institutionalised mongols in Texas *J Ment Defic Res 17* 117 1973
4 Fabia J & Drolette M Life tables up to age 10 for mongols with and without congenital heart defect *J Ment Defic Res 14* 235 1970
5 Forssman H & Åkesson H O Mortality in patients with Down s Syndrome *J Ment Defic Res* 9 146 1965
6 — Note on mortality in patients with Down s Syndrome *J Ment Defic Res 11* 106 1967
7 — Mortality of the mentally deficient A study of 12 903 institutionalised subjects *J Ment Defic Res 14* 276 1970
8 Krugman S & Giles J P Viral hepatitis *JAMA 212* 1019 1970
9 Miller R W Down s Syndrome (Mongolism) other congenital malformations and cancers among the sibs of leukemic children *N Engl J Med 268* 393 1963
10 — Neoplasia and Down s Syndrome *Ann NY Acad Sci 171* 637 1970
11 — Relation between cancer and congenital malformations *Isr J Med Sci* 7 1461 1971
12 Record R G & Smith A Incidence mortality and sex distribution of mongoloid defectives *Br J Prev Soc Med* 9 10 1955
13 Skinhøj Dietrichson O Dyggve H Mikkelsen M Petersen P & Stene J Hepatitis and hepatitis associated antigen (HAA) in Down s Syndrome *J Ment Defic Res 15* 236 1971
14 Sutnick A I London W T Gerstley B J S Cronlund M M & Blumberg B S Anicteric hepatitis associated with Australia antigen *JAMA 205* 670 1968
15 Szmuness W Pick R & Prince A M The serum hepatitis virus specific antigen (SH) A preliminary report of epidemiologic studies in an institution for the mentally retarded *Am J Epidemiol 92* 51 1970
16 Øster J *Mongolism* Danish Science Press Copenhagen 1953
17 Øster J Mikkelsen M & Nielsen A The mortality and causes of death in patients with Down s Syndrome (mongolism) *Proc Int Copenhagen Congr Sci Study Ment Retard I* 410 1964

The following sources have been used in the statistical evaluation

18 Befolkningens Bevægelser 1970 *Statistiske Meddelelser* 7 Table 22 1972 Danmarks Statistik København
19 *Dødsårsagerne i Danmark 1955* Table 8 Sundhedsstyrelsen København 1957
20 *Dødsårsagerne i Danmark i året 1965* Table 11a Sundhedsstyrelse København 1967
21 *Statistisk Årbog 1962* Table 34 Det Statistiske Departement København 1963
22 *Ægteskaber Fødte og Døde 1951–1955* Table XIV Tabelværk *I* 1962 Det Statistiske Departement København 1962

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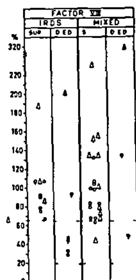


Fig 3 Comparison of F VIII in infants with IRDS and mixed disorders and in surviving and non surviving infants

group and the mixed group and between the surviving and the dead The IRDS group did not differ from the mixed group in maturity ($p>0.05$) Those who had succumbed however had had a lower gestational age than those who had survived ($p<0.001$)

P&P (Fig 2) In the total IRDS group the values ranged between 8% and 45%

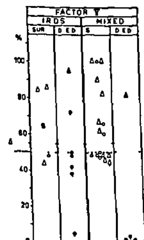


Fig 4 Comparison of F V in infants with IRDS and mixed disorders and in surviving and non surviving

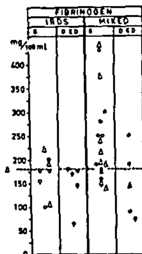


Fig 5 Comparison of fibrinogen in infants with IRDS and mixed disorders and in surviving and non surviving infants

(mean 26% S D ±9 6%) and in the total mixed group between 10% and 65% (mean 38% S D ±12%) This difference was significant ($p<0.001$) The difference in the values between those who survived (range 10–65% mean 35% S D ±11%) and those

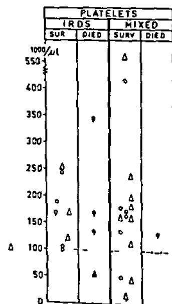


Fig 6 Comparison of platelet counts in infants with IRDS and mixed disorders and in surviving and non surviving infants

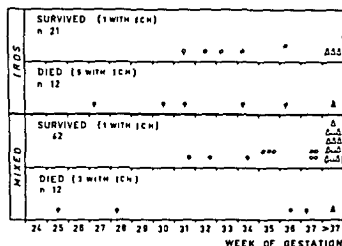


Fig 1 Survey of material Circles denote preterm infants triangles term infants Unfilled symbols denote survivors filled symbols those who died A vertical on a symbol denotes intracranial haemorrhage

department and cared for in the same neonatal unit One infant was given whole blood due to haemorrhagic shock

Blood sampling

Blood specimens were obtained 2 to 24 hours after delivery from an indwelling plastic catheter inserted in one of the umbilical arteries for therapeutic and diagnostic purposes The blood was collected with the silicone technique and citrated plasma was prepared as described previously (32)

Owing to the general condition and smallness of the infants as well as technical difficulties it was not always possible to obtain sufficient blood for a complete set of determinations The determinations made are given in Table I

LABORATORY METHODS

Platelet counts were made by the method of Bjorkman

Table I *Laboratory studies in entire material (87 infants)*

Determination	Infants examined
Factor VIII	72
P&P	53
Factor V	65
Fibrinogen	65
Platelets	62
Fibrin/fibrinogen degradation products	82
Plasminogen	81
Fibrin plates	28
Ethanol gelation	10

(6) Prothrombin factor VII and factor X were measured by the P&P method of Owren & Aas (56)

Factor V activity was determined according to Wolf (65) and factor VIII according to Nilsson et al (51)

Fibrinogen was measured by an immunochemical method of Karaca et al (31) Plasminogen was determined by an immunochemical method according to Ganrot & Niléhn (22)

Fibrinolytic activity of plasma was measured on unheated bovine fibrin plates described by Nilsson & Olow (52)

Fibrin/fibrinogen degradation products (FDP) were determined with the immunochemical method of Niléhn (50) The determinations of FDP were performed on serum obtained from blood collected in tubes containing thrombin and EACA

The ethanol gelation test described by Godal & Abildgaard (23) was used as a test for fibrin monomers

Statistical procedures

The significance of differences between means was estimated with Student s *t* test Significances of differences between the factors in various diagnostic groups were studied with the χ^2 test

RESULTS

The normal ranges of the various factors are based on a survey given by Bleyer et al (7) and on personal studies (28) The factor levels were compared between the IRDS

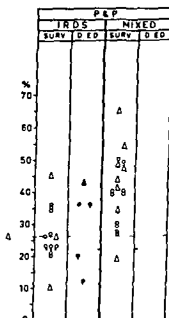


Fig 2 Comparison of P&P in infants with IRDS and mixed disorders and in surviving and non surviving infants Dashed and dotted lines denote lower limits in healthy term and preterm infants

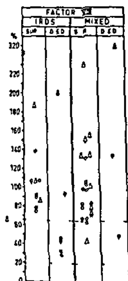


Fig 3 Comparison of F VIII in infants with IRDS and mixed disorders and in surviving and non surviving infants

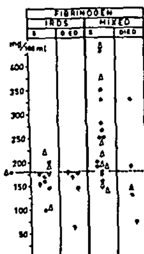


Fig 5 Comparison of fibrinogen in infants with IRDS and mixed disorders and in surviving and non surviving infants

group and the mixed group and between the surviving and the dead The IRDS group did not differ from the mixed group in maturity ($p>0.05$) Those who had succumbed however had had a lower gestational age than those who had survived ($p<0.001$)

P&P (Fig 2) In the total IRDS group the values ranged between 8% and 45% (mean 26% S D ±9 6%) and in the total mixed group between 10% and 65% (mean 38% S D ±12%) This difference was significant ($p<0.001$) The difference in the values between those who survived (range 10–65% mean 35% S D ±11%) and those

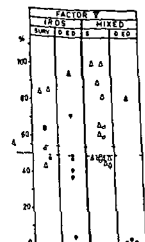


Fig 4 Comparison of F V in infants with IRDS and mixed disorders and in surviving and non surviving infants

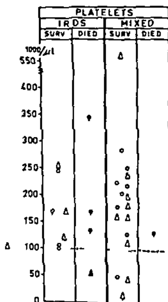


Fig 6 Comparison of platelet counts in infants with IRDS and mixed disorders and in surviving and non surviving infants

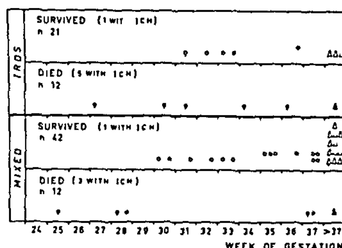


Fig 1 Survey of material Circles denote preterm infants triangles term infants Unfilled symbols denote survivors filled symbols those who died A vertical on a symbol denotes intracranial haemorrhage

department and cared for in the same neonatal unit One infant was given whole blood due to haemorrhagic shock

Blood sampling

Blood specimens were obtained 2 to 24 hours after delivery from an indwelling plastic catheter inserted in one of the umbilical arteries for therapeutic and diag nostic purposes The blood was collected with the silicone technique and citrated plasma was prepared as described previously (32)

Owing to the general condition and smallness of the infants as well as technical difficulties it was not always possible to obtain sufficient blood for a complete set of determinations The determinations made are given in Table 1

LABORATORY METHODS

Platelet counts were made by the method of Bjorkman (6) Prothrombin factor VII and factor X were measured by the P&P method of Owren & Aas (%)

Factor V activity was determined according to Wolf (65) and factor VIII according to Nilsson et al (51)

Fibrinogen was measured by an immunochemical method of Karaca et al (31) Plasminogen was deter mined by an immunochemical method according to Ganrot & Niléhn (22)

Fibrinolytic activity of plasma was measured on un heated bovine fibrin plates described by Nilsson & Olow (52)

Fibrin/fibrinogen degradation products (FDP) were determined with the immunochemical method of Niléhn (50) The determinations of FDP were performed on serum obtained from blood collected in tubes con taining thrombin and EACA

The ethanol gelation test described by Godal & Abildgaard (23) was used as a test for fibrin monomers

Table 1 *Laboratory studies in entire material (87 infants)*

Determination	Infants examined
Factor VIII	72
P&P	53
Factor V	65
Fibrinogen	65
Platelets	6?
Fibrin/fibrinogen degradation products	82
Plasminogen	81
Fibrin plates	28
Ethanol gelation	10

Statistical procedures

The significance of differences between means was estimated with Student s *t* test Significances of differences between the factors in various diagnostic groups were studied with the χ^2 test

RESULTS

The normal ranges of the various factors are based on a survey given by Bleyer et al (7) and on personal studies (28) The factor levels were compared between the IRDS

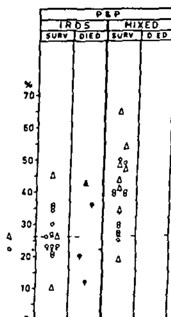


Fig 2 Comparison of P&P in infants with IRDS and mixed disorders and in surviving and non surviving infants Dashed and dotted lines denote *lower limits* in healthy term and preterm infants

Table 4 *Abnormal findings in infants examined*

	Total no of infants	F VIII	P&P	F V	Fibrinogen	Platelets	FDP	Fibrinolysis	Plasminogen
IRDS	?7	0/?6	6/?0	10/?4	16/ 1	?/21	10/?7	4/9	0/?7
Mixed	50	1/40	./?9	17/35	9/36	5/35	1?/45	4/16	0/46
ICH	10	0/6	?/4	5/6	6/8	1/6	9/10	?/3	0/8
Abnormal findings %		1	19	49	48	13	38	36	0

(28 55)) No difference was found between the IRDS group (range 54 000–344 000/μl mean 177 000/μl S D ±67 000/μl) and the mixed group (range 14 000–560 000/μl mean 175 000/μl S D ±104 000/μl) ($p>0\ 05$) or between the surviving (range 14 000–560 000/μl mean 181 000/μl S D ±89 000/μl) and the dead (range 40 000–344 000/μl mean 147 000/μl S D ±92 000) ($p>0\ 05$)

Fibrin/fibrinogen degradation products (FDP) In the whole material FDP (more than 5 μg/ml serum=FDP positive) were found in 38% (Table 2) In the IRDS group 15 out of 33 infants examined were FDP positive (45%) and in the mixed group 16 out of 49 were FDP positive (33%) This difference was not significant ($p>0\ 05$) In the surviving group FDP were positive in 14 out of 59 infants (24%) while 17 out of the 23 who had died were positive (74%) This difference was significant ($p<0\ 01$) Irrespective of the diagnostic group FDP were found in 9 out of the 10 infants with ICH

The amounts of FDP were small (less than 10 μg/ml) or moderate (11–60 μg/ml) in 29

Table 5 *Multiple defects in infants in whom 2 or more of the determinations of P&P F V fibrinogen FDP and platelets had been made*

	IRDS		Mixed		Total	
	Surv	Died	Surv	Died	Surv	Died
	8/19	11/1	8/38	6/8	16/57	17/20
ICH	1/1	5/5	1/1	1/	/?	6/7
%	61		30		28	85

out of 31 infants In 2 infants the FDP levels were very high (Tables 2 and 3)

Fibrinolytic activity in plasma was estimated in 28 infants The fibrinolytic activity was high (>85 mm) (18) in 5 infants with IRDS and 5 with mixed disorders Of these 10 infants of whom 7 died the fibrinogen values were below normal in 4 and FDP were positive in 5

Plasminogen (Fig 7) In the total IRDS group the values ranged between 17% and 45% (mean 26% S D ±6%) and in the total mixed group between 15% and 60% (mean 32% S D ±12%) The difference was significant ($p<0\ 005$) The values in the total surviving group (range 15–60% mean 31% S D ±11%) compared with the values in those who died (range 10–40% mean 25% S D ±7%) also differed significantly ($p<0\ 01$) The comparison between the dead and the surviving within the IRDS group showed no significant difference ($p>0\ 05$)

Ethanol gelation test for fibrin monomers was performed in 10 infants all with negative results Two of these infants had low fibrinogen One showed high fibrinolytic activity and FDP were demonstrated in 2

Some changes were strikingly more common Thus in the whole material factor V was low in 49% fibrinogen was low in 48% and FDP were demonstrated in 38% while factor VIII was abnormally low in only 1% P&P in 19% and platelets in 13% No abnormally low plasminogen values were recorded (Table 4) FDP were found in most of the infants who died (74%) 9 of the 10 infants with ICH proved to have FDP (Table 2)

Table 2. *Positive FDP/infants examined*

Amounts of FDP (µg/ml): 5–10: 11 *infants*; 11–60: 18 infants; >300: 2 infants

	Survived	Died	Total
IRDS	6/21	9/12	15/33
Mixed	8/38	8/11	16/49
Total	14/59	17/23	31/82
ICH	2/2	7/8	9/10

who died (range 8–42%, mean 23%, S.D. ± 11%) was also significant ($p<0.005$).

F VIII (Fig. 3). In the total IRDS group the values ranged between 29% and 200% (mean 95%, S.D. ±48%) and in the total mixed group between 45% and 320% (mean 117%, S.D. ±64%). The values did not differ significantly ($p>0.05$). Neither did the values in the total surviving group (range 45–230%, mean 106%, S.D. ±42%) differ from those obtained in the infants who had died (range 29–320%, mean 116%, S.D. ±90%) ($p>0.05$).

F V (Fig. 4). In the total IRDS group the factor V levels ranged between 5% and 100% (mean 57%, S.D. ±23%) and in the total mixed group between 0% and 107% (mean 55%, S.D. ±27%). There was no significant difference between the two groups ($p>0.05$). A difference was found between the infants who had survived (range 10–107%, mean 60%, S.D. ±21%) and those who had died (range 0–100%, mean 44%, S.D. ±33%) ($p<0.025$).

Fibrinogen (Fig. 5). A highly significant difference was found both between the total IRDS group (range 0.02–0.22 g/100 ml, mean 0.14 g/100 ml, S.D. ±0.06 g/100 ml) and the total mixed group (range 0.07–0.44 g/100 ml, mean 0.23 g/100 ml, S.D. ±0.10 g/100 ml) ($p<0.001$) and the survivors (range 0.06–0.44 g/100 ml, mean 0.22 g/100 ml, S.D. ±0.09 g/100 ml) and those who had died (range 0.02–0.33 g/100 ml, mean 0.13 g/100 ml, S.D. ±0.08 g/100 ml) ($p<0.001$).

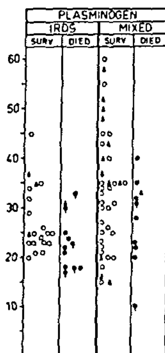


Fig. 7. Comparison of plasminogen in infants with IRDS and mixed disorders and in surviving and non-surviving infants.

Platelet counts (Fig. 6). Of the entire material only 8 infants were thrombocytopenic (i.e. platelet counts below 100000/µl

Table 3. *Illustrative cases*

No.	Gest. age week	Birth weight	Clinical data	Age at death hrs	F VIII (%)	P&P (%)	F V (%)	Fibrinogen (mg/100 ml)	Platelets (×1000/µl)	FDP (µg/ml)
1	27	1 000	Premat. rupt. membr. IRDS severe acidosis/hypoxia	8	33	8 ↓	25 ↓	45 ↓	–	300 ↑
2	37	2 070	Ablatio plac. perinatal asphyxia multiple haemorrh.	33	50	–	3 ↓	75 ↓	132	400 ↑
3	30	1 200	Premat. rupt. membr. IRDS ICH	5	90	70 ↓	5 ↓	63 ↓	134	20 ↑
4	40	2 910	IRDS Mb. down	Survived	188	10 ↓	44 ↓	105 ↓	177	25 ↑

Proof of a condition with abnormal proteolysis requires demonstration of an abnormal degradation of fibrinogen The fibrinogen levels may be low but utilisation of fibrinogen could well be compensated for by a high regenerating capacity (3 32) The action of thrombin on fibrinogen results in the formation of soluble fibrin monomers which can be demonstrated with the ethanol gelation test (23) Some investigators have found this test to be reliable and useful in DIC in humans (34 35) and in experimental DIC in animals (5 60) while other authors question its value in the diagnosis and effect of therapy in a given patient (16 27 45) Other tests for estimating degraded fibrinogen such as fibrinogen chromatography (20) the GEE ^{14}C method of Kisker & Rush (37 38) for determining circulating soluble fibrin and tests regarding fibrinopeptide A (53) are still under evaluation

Fibrinolytic activators in the circulation are difficult to demonstrate because of the shortness of their half lives (21) It is also important to consider the fairly rapid loss of fibrinolytic activity from plasma after the blood sample has been drawn (19)

Low levels of plasminogen and very high levels of FDP usually provide good evidence of a fibrinolytic state Determination of plasminogen in this age is however not very informative since the values in the preterm infant are low and have a wide range of variations (17)

Cases 1 and 2 illustrate abnormal proteolysis leading mainly to activation of the fibrinolytic system as indicated by the low fibrinogen levels and very large amounts of FDP while in cases 3 and 4 both the fibrinolytic and the coagulation systems had been activated (Table 3) All 4 infants had a platelet count above 100000/μl which in our opinion cannot be regarded as thrombocytopenia of clinical importance

Hathaway et al (26) and Altstatt et al (1) stressed the frequent occurrence of DIC in sick newborns and especially its close association with IRDS Markarian et al (47) postulated DIC to be an important pathogenetic factor in IRDS and found a close correlation between hypercoagulability in cord blood and subsequent development of hyaline membranes Chessells & Wigglesworth (12) and Hurlet Birk Jensen et al (29) believe the coagulation changes to be secondary to the neonatal disorder Micro techniques allowing multiple factor analyses and serial estimations have been used by Hurlet Birk Jensen et al (29) and Stuart et al (63) Confirmation of the reproducibility of the assays requires further investigation

Since DIC is believed to play a pathogenetic role in sick newborns heparin has been recommended in the treatment of defective coagulation in such infants Several case reports describing infants recovering during treatment with heparin may however lead to unfounded overestimation of the value of this drug In certain fatal cases heparin may have contributed to the fatal bleedings Klein & Robboy (39) described a newborn who had died from intracranial haemorrhage during heparin therapy In that case it cannot be excluded that heparin had contributed to the fatal course Controlled studies on heparin treatment are still lacking Markarian et al (48) did not show any improvement in the mortality rate or the incidence of post mortem findings of hyaline membranes and Corrigan & Jordan (13) did not find the mortality to be lower in heparin treated septicaemias A recent report by Corrigan et al (14) of 3 cases with septic shock not treated with heparin supports this reluctance to the use of heparin

In general our laboratory findings compare well with the results obtained by others Thus the most frequent abnormalities were low fibrinogen factor V and raised FDP (Table 4) We consider the multiple defects found in our material in both the IRDS group and the mixed group to be secondary to and vary with the severity

ILLUSTRATIVE CASES (Table 3)

Case 1

A boy with a b w of 1000 g born in the 27th week of gestation after premature rupture of the membranes Breech delivery Apgar score at 1 5 10 minutes was 2 Developed IRDS with severe hypoxia and acidosis Died 8 hours after delivery Blood obtained 6 5 hours after birth contained P&P 8% F VIII 33% fibrinogen 0 05 g/100 ml and FDP 300 µg/ml Autopsy showed hyaline membranes in the alveoli No haemorrhage

Case 2

A girl with a b w of 2070 g born in 37th week of gestation after a few days maternal bleeding due to abruptio placentae She was small for *gestational age* Complicated instrumental delivery She was seriously depressed at birth and needed resuscitation She was anaemic and in shock 20 ml bank blood and buffer were given which resulted in slight improvement At the age of 12 hours she had convulsions and spells of apnoea Cerebrospinal fluid contained blood She died 33 hours after delivery Blood obtained 7 hours after birth contained factor VIII 50% factor V 3% fibrinogen 0 08 g/100 ml platelets 132 000/µl plasminogen 10% and FDP 400 µg/ml Autopsy revealed widespread bleeding in the brain meninges connective tissues liver kidneys adrenals gut uterus and also several haemorrhagic necroses in the cerebral hemispheres the liver kidneys and adrenals The vessels in these necroses but not in other areas contained abundant fibrin deposits

Case 3

A boy with a b w of 1200 g born in the 30th week of gestation after premature rupture of the membranes Normal delivery Moderately depressed at birth Apgar scores at 1 5 and 10 minutes 6 *Developed grave respiratory* insufficiency and died 5 hours after delivery P&P was 20% factor VIII 90% factor V 5% fibrinogen 0 06 g/100 ml platelets 134 000/µl and FDP 20 µg/ml Autopsy showed hyaline membranes in the lungs and intraventricular haemorrhage

Case 4

A boy born at term weighing 2910 g Breech delivery Apgar at 1 and 5 minutes 2 and 5 respectively Developed clinical and roentgenological signs of IRDS A moderate hypoxia and acidosis well compensated by treatment with oxygen and buffer Blood obtained 6 hours after delivery showed P&P 10% factor VIII 88% factor V 44% fibrinogen 0 11 g/100 ml platelet count 172 000/µl and FDP 25 µg/ml

DISCUSSION

In the present material of sick newborns multiple defects (i e at least 2 abnormal findings in infants in whom two or more of the determinations of P&P, F V fibrinogen fibrin/fibrinogen degradation products and platelets had been made) were found in 28% of the survivors and 85% of those who had succumbed Multiple defects were twice as common in the total IRDS group as in the mixed group (61% and 30% respectively) (Table 5) Neither group showed a specific pattern of defects The infants with IRDS were more seriously ill as is reflected by their higher mortality rate (36% compared with 22% in the mixed group) The findings in the present material suggested the following assumptions (1) haemostatic defects are not more common in IRDS than in other neonatal disorders of similar severity (2) the defects in IRDS do not differ from those in other neonatal disorders (3) multiple defects are connected with a poor prognosis

Multiple defects of the haemostatic mechanism are commonly regarded as signs of disseminated intravascular coagulation (DIC) or defibrination syndrome DIC may be described as an activation of the coagulation process in vivo by formation of thrombin in the circulating blood with consumption of platelets fibrinogen and other coagulation factors and deposition of fibrin within small vessels DIC is followed by an activation of the fibrinolytic system with the appearance of fibrin/fibrinogen degradation products in serum Occasionally the defibrination results in haemorrhages

In the interpretation of multiple defects it is essential to realise that the results though similar may reflect different mechanisms such as (1) impaired synthesis due to vitamin K deficiency and/or liver damage (43 44) (2) abnormal activity of proteolytic enzymes liberated by tissue damage causing (*a*) activation of the coagulation process (*b*) an activation of the fibrinolytic system or (*c*) a combined activation of both the coagulation and the fibrinolytic systems A more appropriate term for these latter conditions would be disorders with pathologic proteolysis

seminated intravascular coagulation in septic shock Report of three cases not treated with heparin *Am J Dis Child 126* 6 9 1973
deSa D J & Nicholls S Haemorrhagic necrosis of the adrenal gland in perinatal infants a clinico pathologic study *J Pathol 106* 133 1972
Deykin D The clinical challenge of disseminated intravascular coagulation *N Engl J Med 283* 636 1970
Ekelund H & Finnstrom O Fibrinolysis in pre term infants and in infants small for gestational age *Acta Paed at Scand 61* 185 1972
Ekelund H Hedner U & Nilsson I M Fibrinolysis in newborns *Acta Paediatr Scand 59* 33 1970
Fearnley G R *Fibrinolysis* Edw Arnold Ltd London 1965 p 48
Fletcher A P Al jaersig N Roy L & Owens O Early detection of blood hypercoagulable states and early thromboembolic lesions in man *Coll q um on A J A* Brussels Belgium May 7 1972 p 1
Fletcher A P Biederman O Moore D Alkjaersig N & Sherry S Abnormal plasminogen plasmin system activity (fibrinolysis) in patients with hepatic cirrhosis its cause and consequences *J Clin Invest 43* 681 1964
Ganrot P O & Nilehn J E Immunochemical determination of human plasminogen *Clin Chim Acta 2?* 35 1968
Godal H C & Abildgaard U Gelation of soluble fibrin in plasma by ethanol *Scand J Haematol 3* 342 1966
Gralnick H R & Greipp P Thrombo is with epsilon aminocaproic acid therapy *Am J Clin Pathol 56* 151 1971
Gray O P Ackerman A & Fraser A J Intracranial haemorrhage and clotting defects in low birth weight infants *Lancet I* 545 1968
Hathaway W E Mull M M & Pechet G S Disseminated intravascular coagulation in the new born *Pediatrics 43* 233 1969
Hedner U & Nilsson I M Parallel determinations of FDP and fibrin monomers with various methods *Thromb Diath Haemorrh* (Stuttg) *28* 68 1972
Holmberg L Henriksson P Ekelund H & Åstedt B Coagulation in the human fetus Comparison with fullterm newborns *J Pediat* In press
Hurlet Birk Jensen A Josso F Zamet P Monset Couchard M & Minkowski A Evolution of blood clotting factor levels in premature infants during the first 10 days of life a study of 96 cases with comparison between clinical status and blood clotting factor levels *Pediatr Res 7* 638 1973
Hutchison J H Kerr M M Douglas T A Inall J A & Crosbie J C A therapeutic approach in 100 cases of the respiratory distress syndrome of the newborn infant *Pediatrics 33* 956 1964
31 Karaca M Nilsson I M & Hedner U Quantitative determination of platelet fibrinogen *J Lab Clin Med 77* 485 1971
32 Kanitzky D Kleine N Pringsheim W & Kunzer W Fibrinogen turnover in the premature infant with and without idiopathic respiratory distress syndrome *Acta Paed itr Scand 60* 465 1971
33 Karpatkin M Diagnosis and management of disseminated intravascular coagulation *Pediatr Clin North Am 18* 23 1971
34 Kierulf P & Godal H C Fibrinaemia in medical patients screened by the ethanol gelation test *Acta Med Scand 190* 185 1971
35 — Fibrinaemia and multiple thrombi in pancreatic carcinoma *Scand J Haematol 9* 370 1972
36 King J B Some recent advances in coagulation—1972 *S Afr Med J 46* 2085 1972
37 Kisker C T & Rush R Detection of intravascular coagulation *J Clin Invest 50* 2235 1971
38 — Circulating fibrin in meningococcemia *J Pediatr 82* 787 1973
39 Klein R & Robboy S J Thrombocytopenia in a newborn girl (case records of the Massachusetts General Hospital) *N Engl J Med 288* 363 1973
40 Kunzer W Die Blutgerinnung bei Neugeborenen und ihre Störungen *Klin Wochenschr 49* 1 1971
41 Ludwig H Mikrozirkulationsstörungen und Diapedesblutungen im Fetalen Gehirn bei Hypoxie *Bibl Gynaec 46* 172 1968
42 Mahasandana C & Hathaway W E Circulating anticoagulants in the newborn relation to hypercoagulability and the idiopathic respiratory distress syndrome *Pediatr Res 7* 670 1973
43 Mangurten H H & Honig G R Hemorrhagic disease due to vitamin K deficiency in a premature infant a syndrome which may resemble disseminated intravascular coagulation (DIC) *Clin Pediatr 12* 372 1973
44 Mant M J Hirsch J Pineo G F & Luke K H Prolonged prothrombin time and partial thromboplastin time in disseminated intravascular coagulation not due to deficiency of factors V and VIII *Br J Haematol 24* 725 1973
45 Margolis C Z Ethanol gelation test *N Engl J Med 284* 53 1971
46 Margolis C Z Orzalesi M M & Schwartz A D Disseminated intravascular coagulation in the respiratory distress syndrome *Am J Dis Child 125* 324 1973
47 Markarian M Githens J H Rosenblut E Fernandez F Jackson J J Bannon A E Lindley A Lubchenco L O & Martorell R Hypercoagulability in premature infants with special reference to the respiratory distress syndrome and hemorrhage I Coagulation studies *Biol Neonate 17* 84 1971
48 Markarian M Lubchenco L O Rosenblut E Fernandez F Lang D Jackson J J Bannon A E Lindley A Githens J H & Martorell R Hypercoagulability in premature infants with special reference to the respiratory distress syndrome and hemorrhage II The effect of heparin *Biol Neonate 17* 98 1971

of the disorder We believe that in most of our infants the haemostatic derangements were caused by a combination of an abnormal proteolysis acting both as thrombin and plasmin and impaired synthesis of factors due to hypoperfusion and/or tissue hypoxia Therapy must therefore be directed above all to the underlying disorder

In conditions with abnormal degradation of fibrinogen it may be therapeutically important to differentiate between those cases in which an activation of the coagulation process is predominant and those in which an activation of the fibrinolytic system is predominant i e whether the basic treatment should be extended to include heparin or a fibrinolytic inhibitor should be given The use of heparin alone in the treatment of sick newborns with multiple coagulation defects must therefore be questioned We decided not to treat our infants with heparin because of the risk of bleeding complications It is also important to recall that heparin acts prophylactically i e stops further coagulation and has no effect on existing damage

In infants where the disorder has induced activation of the fibrinolytic system mainly fibrinolytic inhibitors such as epsilon aminocaproic acid (EACA) tranexamic acid (AMCA) and Trasylol must be considered Many authors feel that these agents might be dangerous in blocking the fibrinolytic defence mechanism against the deposition of fibrin (26 36 58 59 62) However clinical investigations by Becker et al (4) Gralnick & Greipp (24) and Vinnicombe & Shuttleworth (64) did not show any increased incidence of thrombosis in connection with epsilon aminocaproic acid therapy In animal experiments Åstedt & Liedholm (66) have shown that the activator content of the vessel walls, which is the most important factor in the fibrinolytic defence mechanisms is not influenced by even an intense and prolonged blockade of the fibrinolytic system Antifibrinolytic therapy as suggested by Ludwig (41) in combination with fresh blood or plasma in cases with suspected bleeding and increased fibrinolysis may therefore be adequate

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REFERENCES

1 Altstatt L B Dennis L H Sundell H Malan A Harrison V Hedvall G Eichelberger J Fogel B & Stahlman M Disseminated intravascular coagulation in hyaline membrane disease Biol Neonate 19 227 1971
2 Ambrus C M Ambrus J L Weintraub D H Foote R J Courey N G & Niswander K R Thrombolytic therapy in hyaline membrane disease Thromb Diath Haemorrh Suppl 47 269 1971
3 Baker L R I Rubenberg M L Dacie J V & Brain M C Fibrinogen catabolism in microangiopathic haemolytic anaemia Br J Haematol 14 617 1968
4 Becker J Borgstrom S & Saltzman G F Incidence of thrombosis associated with epsilon aminocaproic acid administration and with combined epsilon aminocaproic acid and subcutaneous heparin therapy Acta Chir Scand 136 167 1970
5 Beller F K & Theiss W Fibrin derivatives Plasma hemoglobin and glomerular fibrin deposition in experimental intravascular coagulation Thromb Diath Haemorrh (Stuttg) 29 363 1973
6 Bjorkman S E A new method for enumeration of platelets Acta Haematol 22 377 1959
7 Bleyer W A Hakami N & Shepard T H The development of hemostasis in the human fetus and newborn infant J Pediatr 79 838 1971
8 Chadd M A Elwood P C Gray O P & Muxworthy S M Coagulation defects in hypoxic fullterm newborn infants Br Med J 4 516 1971
9 Chessells J M & Wigglesworth J S Secondary haemorrhagic disease of the newborn Arch Dis Child 45 539 1970
10 — Haemostatic failure in babies with rhesus isoimmunization Arch Dis Child 46 38 1971 a
11 — Coagulation studies in severe birth asphyxia Arch Dis Child 46 253 1971 b
12 — Coagulation studies in preterm infants with respiratory distress and intracranial haemorrhage Arch Dis Child 46 564 1972
13 Corrigan J J & Jordan C M Heparin therapy in septicemia with disseminated intravascular coagulation Effect on mortality and on correction of haemostatic defects N Engl J Med 283 778 1970
14 Corrigan J J Jordan C M & Bennet B B Dis-

seminated intravascular coagulation in septic shock Report of three cases not treated with heparin *Am J Dis Child 126* 6 9 1973

5 deSa D J & Nicholls S Haemorrhagic necrosis of the adrenal gland in perinatal infants a clinico pathologic study *J Pathol 106* 133 1972

16 Deykin D The clinical challenge of disseminated intravascular coagulation *N Engl J Med* 83 636 1970

17 Ekelund H & Finnstrom O Fibrinolysis in pre term infants and in infants small for gestational age *Acta Paediatr Scand 61* 185 1972

18 Ekelund H Hedner U & Nilsson I M Fibrinolysis in newborns *Acta Paediatr Scand 59* 33 1970

19 Fearnley G R *Fibrinolysis* Edw Arnold Ltd London 1965 p 48

20 Fletcher A P Alkjaersig N Roy L & Owens O Early detection of blood hypercoagulable states and early thromboembolic lesions in man *Colloquum on A J A* Brussels Belgium May 2 1972 p 1

21 Fletcher A P Biederman O Moore D Alkjaersig N & Sherry S Abnormal plasminogen plasmin system activity (fibrinolysis) in patients with hepatic cirrhosis its cause and consequences *J Clin Invest 43* 681 1964

22 Ganrot P O & Niléhn J E Immunochemical determination of human plasminogen *Clin Chim Acta 22* 235 1968

23 Godal H C & Abildgaard U Gelation of soluble fibrin in plasma by ethanol *Scand J Haematol 3* 34 1966

24 Gralnick H R & Greipp P Thrombosis with epsilon aminocaproic acid therapy *Am J Clin Pathol 56* 151 1971

25 Gray O P Ackerman A & Fraser A J Intracranial haemorrhage and clotting defects in low birth weight infants *Lancet I* 545 1968

26 Hathaway W E Mull M M & Pechet G S Disseminated intravascular coagulation in the newborn *Pediatrics 43* 233 1969

27 Hedner U & Nilsson I M Parallel determinations of FDP and fibrin monomers with various methods *Thromb Diath Haemorrh* (Stuttg) *28* 268 1972

28 Holmberg L Henriksson P Ekelund H & Åstedt B Coagulation in the human fetus Comparison with fullterm newborns *J Pediatr* In press

29 Hurlet Birk Jensen A Josso F Zamet P Monset Couchard M & Minkowski A Evolution of blood clotting factor levels in premature infants during the first 10 days of life a study of 96 cases with comparison between clinical status and blood clotting factor levels *Pediatr Res* 7 638 1973

30 Hutchison J H Kerr M M Douglas T A Inall J A & Crosbie J C A therapeutic approach in 100 cases of the respiratory distress syndrome of the newborn infant *Pediatrics 33* 956 1964

31 Karaca M Nilsson I M & Hedner U Quantitative determination of platelet fibrinogen *J Lab Clin Med 77* 485 1971

32 Kanitzky D Kleine N Pringsheim W & Kunzer W Fibrinogen turnover in the premature infant with and without idiopathic respiratory distress syndrome *Acta Paediatr Scand 60* 465 1971

33 Karpatkin M Diagnosis and management of disseminated intravascular coagulation *Pediatr Clin North Am 18* 23 1971

34 Kierulf P & Godal H C Fibrinaemia in medical patients screened by the ethanol gelation test *Acta Med Scand 190* 185 1971

35 — Fibrinaemia and multiple thrombi in pancreatic carcinoma *Scand J Haematol 9* 370 197

36 King J B Some recent advances in coagulation— 197 *S Afr Med J 46* 085 197

37 Kisker C T & Rush R Detection of intravascular coagulation *J Clin Invest 50* 2 35 1971

38 — Circulating fibrin in meningococcemia *J Pediatr 82* 787 1973

39 Klein R & Robboy S J Thrombocytopenia in a newborn girl (case records of the Massachusetts General Hospital) *N Engl J Med 288* 363 1973

40 Kunzer W Die Blutgerinnung bei Neugeborenen und ihre Störungen *Klin Wochenschr 49* 1 1971

41 Ludwig H Mikrozirkulationsstörungen und Diapedesblutungen im Fetalen Gehirn bei Hypoxie *Bibl Gynaec 46* 177 1968

42 Mahasandana C & Hathaway W E Circulating anticoagulants in the newborn relation to hypercoagulability and the idiopathic respiratory distress syndrome *Pediatr Res 7* 670 1973

43 Mangurten H H & Honig G R Hemorrhagic disease due to vitamin K deficiency in a premature infant a syndrome which may resemble disseminated intravascular coagulation (DIC) *Clin Pediatr 12* 37 1973

44 Mant M J Hirsch J Pineo G F & Luke K H Prolonged prothrombin time and partial thromboplastin time in disseminated intravascular coagulation not due to deficiency of factors V and VIII *B J Haematol 24* 7 5 1973

45 Margolis C Z Ethanol gelation test *N Engl J Med 284* 53 1971

46 Margolis C Z Orzalesi M M & Schwartz A D Disseminated intravascular coagulation in the respiratory distress syndrome *Am J Dis Child 125* 324 1973

47 Markarian M Githens J H Rosenblut E Fernandez F Jackson J J Bannon A E Lindley A Lubchenco L O & Martorell R Hypercoagulability in premature infants with special reference to the respiratory distress syndrome and hemorrhage I Coagulation studies *Biol Neonate 17* 84 1971

48 Markarian M Lubchenco L O Rosenblut E Fernandez F Lang D Jackson J J Bannon A E Lindley A Githens J H & Martorell R Hypercoagulability in premature infants with special reference to the respiratory distress syndrome and hemorrhage II The effect of heparin *Biol Neonate 17* 98 1971

49 McMillan C W Weiss A E & Johnson A M Acquired coagulation disorders in children *Pediatr Clin North Am 19* 1029 1972
50 Niléhn J E Separation and estimation of split products of fibrinogen and fibrin in human serum *Thromb Diath Haemorrh* (Stuttg) *18* 487 1967
51 Nilsson I M Blomback M & von Francken I On an inherited autosomal haemorrhagic diathesis with antihaemophilic globulin (AHG) deficiency and prolonged bleeding time *Acta Med Scand 159* 35 1957
52 Nilsson I M & Olow B Determination of fibrinogen and fibrinogenolytic activity *Thromb Diath Haemorrh* (Stuttg) *8* 297 1962
53 Nossel H L Canfield R E & Butler V P Plasma fibrinopeptide A concentration as an index of intravascular coagulation *Abstr IV Internat Congr Thrombosis and Haemostasis* Vienna 1973 No 202
54 Oppenheimer E H & Esterly J R Thrombosis in the newborn comparison between infants of diabetic and nondiabetic mothers *J Pediatr 67* 549 1965
55 Oski F A & Naiman J L *Hematologic problems in the newborn* W B Saunders Co Philadelphia London Toronto 2nd ed 1972 p 257
56 Owren P A & Aas K The control of dicumarol therapy and the quantitative determination of prothrombin and proconvertin *Scand J Clin Lab Invest 3* 201 1951
57 Paraskevas M Nilsson I M & Martinsson G A method for determining serum inhibitors of plasminogen activation *Scand J Clin Lab Invest 14* 138 1962
58 Robboy S J Colman R W & Minna J D Pathology of disseminated intravascular coagulation (DIC) Analysis of 26 cases *Hum Pathol 3* 327 1972
59 Sherman L A Wessler S & Avioli L V Therapeutic problems of disseminated intravascular coagulation *Arch Intern Med 132* 446 1973
60 Slaastad R A & Jeremic M The laboratory diagnosis of low graded disseminated intravascular coagulation A study in rabbits *Scand J Haematol 11* 50 1973
61 Stark C R Abramson D & Erkan V Intravascular coagulation and hyaline membrane disease of the newborn *Lancet I* 1180 1968
62 Stefanini M Diffuse intravascular coagulation versus fibrinolysis A clinical and laboratory challenge *Postgrad Med 51* 215 1972
63 Stuart J Picken A M Breeze G R & Wood B S B Capillary blood coagulation profile in the newborn *Lancet II* 1467 1973
64 Vinnicombe J & Shuttleworth K E D Aminocaproic acid in the control of haemorrhage after prostatectomy Safety of aminocaproic acid—a controlled trial *Lancet I* 232 1966
65 Wolf P A modification for routine laboratory use of Stefanini s method of estimating factor V activity in human oxalated plasma *J Clin Pathol 6* 34 1953
66 Åstedt B & Liedholm P Tranexamic acid and fibrinolytic activity of the vessel wall *Experientia 30* 776 1974

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ADDITIONAL DATA ON HEPATIC FUNCTION TESTS IN CYSTIC FIBROSIS

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ABSTRACT Feigelson J Pecau Y Cathelineau L and Navarro J (49 Bd Bessieres 75-Paris XVII France) Additional data on hepatic function tests in cystic fibrosis Acta Paediatr Scand 64 337 1975.—Fifty cystic fibrosis (CF) patients of whom 9 had multilobular cirrhosis were observed regularly for a period of 3 years and various liver function tests indicating cytolysis cholestasis and cellular insufficiency were performed Immunoglobulin and prothrombin were assayed In 9 patients with cirrhosis the tests were generally abnormal Two distinct biochemical patterns of cirrhosis were distinguished one clearly cholestatic and the other of a more cellular type The distinction was made on the basis of the IgA Transferrin ratio and of gamma-glutamyl transpeptidase levels. In the non-cirrhotic patients a temporary increase of cytolysis and cholestasis was observed in 50 % of the cases

KEY WORDS Cystic fibrosis liver function tests cirrhosis

Liver involvement occurs frequently in Cystic Fibrosis (CF) and although without clinical manifestations focal biliary fibrosis is commonly found at post mortem (5 6 9 15 22) This is sometimes complicated by fatty infiltration (16 26 28) or multilobar cirrhosis with or without portal hypertension (12 27) So far no explanation has been offered for the rare incidence of these complications In our series it seemed to be unrelated to the patients age sex or pulmonary condition

The lack of sensitivity of liver function tests (LFTs) has been discussed previously (9) and in this study more sensitive and easily repeatable tests were performed in order to relate hepatocellular disfunction to the appearance of cirrhosis Monitoring LFTs allowed us to observe the onset of viral hepatitis and any subsequent pathology within these abnormal livers

Abbreviations used

LFT=liver function test OTC=ornithine carbamyl transferase B =vitamin B, SGPT=serum glutamic pyruvic transaminase AP=alkaline phosphatase LAP=leucine aminopeptidase γGT=gamma glutamyl transpeptidase IgA Tr=IgA Transferrin

MATERIAL

Fifty patients aged from 1 to 25 years 0 males and 30 females suffering from CF confirmed by a sweat test and with varied and fluctuating pulmonary function were observed carrying out regular LFTs for a period of 3 years At the start of this study 8 patients already showed clinical evidence of cirrhosis and a ninth (no 70) developed it subsequently The diagnosis of cirrhosis was made later due to the emergence of the following criteria hepatosplenomegaly thrombocytopenia radiological demonstration of oesophageal varices and associated liver scan abnormalities In 3 cases (nos 17 27 39) autopsies were performed and showed evidence of multilobar cirrhosis Our material concerning the other cases of cirrhosis is open to discussion We deliberately did not make any liver biopsy as lesions are mainly focal interpretation uncertain and because of the lack of therapeutic value

Of the 9 patients with cirrhosis 4 had oesophageal varices and all had some degree of thrombocytopenia Seven died during the study one from bleeding oesophageal varices (no 29) 5 from respiratory failure (no 17 27 30

Table 1 *Data of biological findings in the cirrhotic patient group*

Obs no	Age	Sex	Stage of study	Oesoph varices	SGPT RFU	OTC (μM/ml)	Vit B_{12} (pg/ml)	AP (IU/l)	LAP (IU/l)	γGT (IU/l)	Albumin (mg/100 ml)	Prealbumin (% of normal)
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	13		Final		90	0 96		170	65	90	4 400	37
19	5	F	First	–	50	0 55	830	100	32	48	3 000	
	7		Final		600	0 85		100	32	42	3 100	[illegible]
27	4	M	First	+	40	1 15	980	92	105	145	4 000	
	6		Final		800	12 00	1 450	260	61	210	4 000	50
29	23	M	First	+	42			250			2 600	
30	9	M	First	+	140	4 60	1 600	128	60	260	4 400	38
			Final		50	0 85		310	32	56	3 200	[illegible]
39	11	F	First	+	60	0 90	1 200	160	60	150	4 800	
	12		Final		60	2 10	1 100	140	60	398	3 800	45
41	12	M	First	–	45	0 65	430	110	26	50	4 [illegible]00	
70	8	F	First	–	35	0 60		110	40	55	3 800	65
100	20	M	Final		200	0 30	430	200	65	55	3 200	10

39 100) but only one patient from hepatic failure with coma (no 19) 41 patients showed no evidence of cirrhosis and of these 3 died from respiratory failure with cardiac livers In one of these (no 36) histology showed fatty infiltration and the autopsies confirmed that none of these 3 patients had multilobar cirrhosis

METHOD

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Tests of cholestasis 3 enzyme activities were studied *alkaline phosphatase* (AP) was measured by the Babson method (2) in International Units This investigation was made but the results interpreted with reservation The upper limit of normal was taken as 100 I U realising the difficulty in comparing the levels of normal subjects with those of CF patients of the same age whose growth is generally affected by various factors such as intestinal malabsorption or severe infections *Leucine aminopeptidase* assay (LAP) was carried out by Nagel s kinetic method (19) on a leucine-*p* nitroanilid substrate (normal values~20 I U /l) *Gammaglutamyl transpeptidase* assay (γGT) was carried out by the kinetic method of Szasz on a L γ glutamyl-*p* nitroanilide substrate (25) the upper limit of normal was taken as 25 I U /l

For LAP and γGT levels age was not taken into account since it was considered significant only during the neo-natal period

Hepatic cellular insufficiency was monitored by measuring levels of *albumin* by electrophoresis (normal range 3 500–5 500 mg/100 ml) *prealbumin* (normal range 60–140% of the average) (14) and *transferrin* (normal range 200–400 mg/100 ml both by radial immunodiffusion) *cholinesterase* by the Ellman kinetic method (8) (normal range using Boehringer reagent 1 900–3 800 I U /l)

Serum prothrombin was measured regularly and clotting factors assayed if it was found to be low (21)

Moreover the levels of *IgA IgG and IgM* were measured serially by radial immunodiffusion and the results corrected for age according to the Geiger & Hoffman table (4 15)

The serial measurement of B S P clearance was abandoned because apart from being repeatedly unacceptable to the patients it proved insensitive to early changes in hepatic function and too dependent on the intrahepatic blood flow (3) The measurement of cholesterol and its ester was also abandoned because of fat malabsorption in cystic fibrosis

RESULTS

In the group of patients with *cirrhosis* (Tables 1 and 2) the cytolysis tests showed consistently raised SGPT and OTC levels with considerable fluctuations particularly terminally Serum B₁₂ levels were also frequently raised (Fig

...ans-...rin ...g/ ...0 ml)	Choline sterase (IU/l)	IgG (mg/100 ml)	IgM	IgA	IgA / Transfer
...60	1 990	2 ?00	180	?70	0 5
...00	585	2 740	376	680	3 4
...5	1 500	2 700	180	450	3 6
...0	935	3 090	170	995	8 ?
...60	? 700	? 000	110	145	0 3
...60	1 400	? 400	190	95	0 3
		Increased			
...10	1 640	? 400	250	175	0 5
...00	1 170	2 00	4 0	242	0 8
	1 755				
...40	1 050	1 650	?20	??0	0 9
...70	? 900	970	?85	470	1 7
...30	? ?00	3 000	?00	63?	1 9
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patients (nos 27 and 41) these increases occurred several months after the appearance of clinical evidence of cirrhosis

The cholestasis tests were always abnormal with correspondingly increased enzyme levels As for γGT despite the small number of cases studied two sub-groups could be recognised based on either considerably (type 1) or moderately (type 2) increased serum levels

The albumin levels fell significantly only in 4 cases (nos 19 29 30 and 100) The fall in the level of prealbumin turned out to be more sensitive and took place earlier being less than 50% of normal in 6 out of 7 patients The level of cholinesterase even when normal at the beginning always fell sharply terminally The transferrin levels were low in 2 patients (nos 19 and 100) and rose in 2 others (nos 17 and 27) during the preliminary stage of the hepatic disease returning to normal later In 4 patients (nos 30 39 41 and 70) they were normal during the whole study

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The *prothrombin* level was generally reduced by about 50% The *proaccelerin* level also fell on average by about 50% in the 4 cases studied It must be noted however that there were considerable variations in these parameters during the course of the disease so that individuals sometimes showed normal levels The lowest prothrombin level recorded was 32% of normal

Using the cytolysis tests in the *non cirrhotic group* excepting for those with viral hepatitis and even when the SGPT were minimally affected 50% of the patients had temporarily elevated OTC levels at some stage of the disease The changes in B_{12} levels followed those of OTC but to a lesser extent Considering the cholestasis tests alkaline phosphatase levels corrected for age (15) since some patients were still growing rose slightly whilst the LAP was higher than normal in 45% of the patients in this group The γGT was normal in 73% of patients but the re t showed fluctuating but moderate increases above the normal As for the cellular insufficiency tests serum albumin prealbumin transferrin and cholinesterase were normal apart from the isolated case of a patient aged 6 whose prealbumin level fell to 45% of normal whilst the cholinesterase level fell sharply to 1 100 units terminally Macroscopically at post mortem in this non cirrhotic pa

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	12		Final		60	2 10	1 100	140	60	388	3 800	45
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Using the cytolysis tests in the *non cirrhotic group* excepting for those with viral hepatitis and even when the SGPT were minimally affected 50% of the patients had temporarily elevated OTC levels at some stage of the disease The changes in B_1 levels followed those of OTC but to a lesser extent Considering the cholestasis tests alkaline phosphatase levels corrected for age (15) since some patients were still growing rose slightly whilst the LAP was higher than normal in 45% of the patients in this group The γGT was normal in 73% of patients but the re t showed fluctuating but moderate increases above the normal As for the cellular insufficiency tests serum albumin prealbumin transferrin and cholinesterase were normal apart from the isolated case of a patient aged 6 whose prealbumin level fell to 45% of normal whilst the cholinesterase level fell sharply to 1 100 units terminally Macroscopically at post mortem in this non cirrhotic pa

Table 1 *Data of biological findings in the cirrhotic patient group*

Obs no	Age	Sex	Stage of study	Oesoph varices	SGPT RFU	OTC (μM/ml)	Vit B_{12} (pg/ml)	AP (IU/l)	LAP (IU/l)	γGT (IU/l)	Albumin (mg/ 100 ml)	Prealbumin (% of norm
17	11	F	First	–	160	6 00	1 900	240	65	130	4 600	
	13		Final		90	0 96		170	65	90	4 400	37
19	5	F	First	–	50	0 55	830	100	32	48	3 000	
	7		Final		600	0 85		100	32	42	3 100	22
27	4	M	First	+	40	1 15	980	92	105	145	4 000	
	6		Final		800	12 00	1 450	260	61	210	4 000	50
29	23	M	First	+	42			250			2 600	
30	9	M	First	+	140	4 60	1 600	128	60	260	4 400	38
			Final		50	0 85		310	32	56	3 700	37
39	11	F	First	+	60	0 90	1 200	160	60	150	4 800	
	12		Final		60	2 10	1 100	140	60	388	3 800	45
41	12	M	First	–	45	0 65	430	110	26	50	4 200	
70	8	F	First	–	35	0 60		110	40	55	3 800	65
100	20	M	Final		200	0 30	430	200	65	55	3 700	10

39 100) but only one patient from hepatic failure with coma (no 19) 41 patients showed no evidence of cirrhosis and of these 3 died from respiratory failure with cardiac livers In one of these (no 36) histology showed fatty infiltration and the autopsies confirmed that none of these 3 patients had multilobar cirrhosis

METHOD

Only tests requiring small volumes of blood were performed All tests were done the same day as samples were taken except for ornithine transcarbamylase (OTC) and vitamin B_{12} assays for which sera were kept at −20 C before being analysed

The following tests were carried out *Tests indicating cytolysis serum glutamic pyruvic transaminase* (SGPT) levels were determined colorimetrically (20) (normal range <35 Reitman Frankel units) *serum ornithine transcarbamylase* (OTC) levels with the results in μmol of citruline released/ml/hour according to the Snodgrass & Parry method (24) (normal range <0 20 μmol) *serum B_{12}* levels were assayed using the Mollin & Anderson method (18) (normal values <800 pg/ml

Tests of cholestasis 3 enzyme activities were studied *alkaline phosphatase* (AP) was measured by the Babson method (2) in International Units This investigation was made but the results interpreted with reservation The upper limit of normal was taken as 100 I U realising the difficulty in comparing the levels of normal subjects with those of CF patients of the same age whose growth is generally affected by various factors such as intestinal malabsorption or severe infections *Leucine aminopeptidase* assay (LAP) was carried out by Nagel s kinetic method (19) on a leucine *p* nitroanilide substrate (normal values~20 I U /l) *Gammaglutamyl transpeptidase* assay (γGT) was carried out by the kinetic method of Szasz on a L γ glutamyl-*p* nitroanilide substrate (25) the upper limit of normal was taken as 25 I U /l

For LAP and γGT levels age was not taken into account since it was considered significant only during the neo natal period

Hepatic cellular insufficiency was monitored by measuring levels of *albumin* by electrophoresis (normal range 3 500–5 500 mg/100 ml) *prealbumin* (normal range 60–140% of the average) (14) and *transferrin* (normal range 200–400 mg/100 ml both by radial immunodiffusion) *cholinesterase* by the Ellman kinetic method (8) (normal range using Boehringer reagent 1 900–3 800 I U /l)

Serum prothrombin was measured regularly and clotting factors assayed if it was found to be low (21)

Moreover the levels of *IgA IgG and IgM* were measured serially by radial immunodiffusion and the results corrected for age according to the Geiger & Hoffman table (4 15)

The serial measurement of B S P clearance was abandoned because apart from being repeatedly unacceptable to the patients it proved insensitive to early changes in hepatic function and too dependent on the intrahepatic blood flow (3) The measurement of cholesterol and its ester was also abandoned because of fat malabsorption in cystic fibrosis

RESULTS

In the group of patients with *cirrhosis* (Tables 1 and 2) the cytolysis tests showed consistently raised SGPT and OTC levels with considerable fluctuations particularly terminally Serum B_{12} levels were also frequently raised (Fig 1) In 2

…ans …rin …g/ …0 ml)	Choline sterase (IU/l)	IgG (mg/100 ml)	IgM	IgA	IgA / Transfer
…0	1 990	2 ?00	180	?70	0 5
…0	585	2 740	376	680	3 4
…5	1 500	2 700	180	450	3 6
…70	935	3 090	170	995	8 ?
…60	? 700	? 000	110	145	0 3
…60	1 400	? 400	190	95	0 3
		Increased			
…10	1 640	400	?50	175	0 5
…00	1 170	? ?00	4?0	24?	0 8
	1 755				
…40	1 050	1 650	??0	??0	0 9
…70	? 900	970	?85	470	1 7
…80	2 ?00	3 000	?00	63?	1 9
…70	510	3 000	300	700	4 1

patients (nos 27 and 41) these increases occurred several months after the appearance of clinical evidence of cirrhosis

The cholestasis tests were always abnormal with correspondingly increased enzyme levels As for γGT despite the small number of cases studied two sub groups could be recognised based on either considerably (type 1) or moderately (type 2) increased serum levels

The albumin levels fell significantly only in 4 cases (nos 19 29 30 and 100) The fall in the level of prealbumin turned out to be more sensitive and took place earlier being less than 50% of normal in 6 out of 7 patients The level of cholinesterase even when normal at the beginning always fell sharply terminally The transferrin levels were low in 2 patients (nos 19 and 100) and rose in 2 others (nos 17 and 27) during the preliminary stage of the hepatic disease returning to normal later In 4 patients (nos 30 39 41 and 70) they were normal during the whole study

The levels of IgG and IgM rose considerably during the final stage whatever the cause of death The variations in IgA seemed more significant when considering the cases with liver abnormalities In 5 of the 9 in the Cirrhotic group the level of IgA rose (nos 17 19 41 70 and 100)

According to Aron (1) the IgA Transferrin ratio (Table 2) indicates the development of cirrhosis if greater than 1 9 Thus taking age into account the ratio was more than 1 9 in 4 cases (type 2 nos 19 41 70 and 100) and less than 1 0 in 4 other cases with considerable cholestasis and particularly raised γGT (type 1 nos 17 27 30 and 39) (Fig 2) In one patient (no 17) a secondary change in this ratio took place 2 months before death from respiratory failure The level of IgA rose from 270 to 680 mg/100 ml whilst transferrin fell from 460 to 200 mg/100 ml so that the IgA Tr ratio changed from 0 5 to 3 4 A slight fall was also noted in the γGT level

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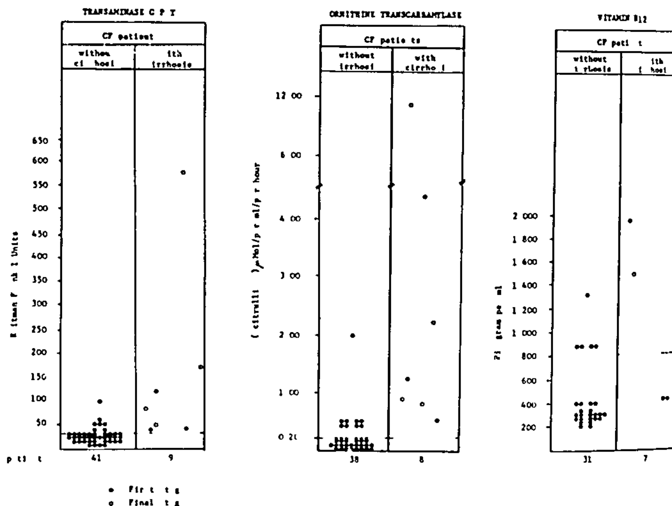


Fig 1 Cytolysis tests in 50 C F patients

tient the liver appeared normal but histological examination carried out in several different areas showed a diffuse fatty infiltration The prothrombin level in 14 of the 41 patients in this group fell temporarily sometimes to as low as 40% Whenever possible any fall in factor V levels was studied and found to be almost parallel to that of prothrombin The variations in the prothrombin level were not generally related to a corresponding change in the enzyme levels In one patient aged 3 months the prothrombin level had fallen to 12% a year before the study began due to intestinal malabsorption of vitamin K_1 and in the course of the study a fall to 35% was observed in another patient of 18 months, where however the factor V level was found to be normal Immunoglobulin levels were generally high particularly IgG probably reflecting a hyperimmune state linked with chronic bronchial infection In 9 patients there was a significant rise in the IgA level

Supervention of viral hepatitis

3 patients (nos 27 34 and 42) one of whom (no 27) already had cirrhosis developed viral hepatitis Two had jaundice and one child had an anicteric form (no 34) This diagnosis was made by serial biochemical investigation the results of which were characteristically abnormal in the whole family affected by the virus The tests for Australia antigen were negative in all cases Patient 42 had a γGT level higher than that normally reached in non CF patients with hepatitis studied at the same time The increase in different enzymes in the cirrhotic patient (no 27) was considerably less than for the other two In this patient the viral hepatitis did not immediately aggravate the state of the

[able 2 *IgA Transferrin ratio corrected ac ·ording to age in the cirrhotic patient group*

)bs ıo	Age	Stage of study	IgA mg/ 100 ml	IgA Trans ferrin
17	11	First	337	0 7
	13	Final	755	3 7
19	5	First	900	7 ?
	7	Final	1 660	13 8
27	4	First	332	0 7
	6	Final	172	0 6
30	9	First	250	0 8
	11	Final	310	1 0
39	1?	Final	?54	1 0
41	12	First	552	2 0
70	8	First	972	? 9
100	20	Final	700	4 1

child and the various tests all resumed their former levels within 2 months 4 months after the onset of hepatitis liver function dete riorated causing a gradual fall in serum cholinesterase The case was terminated by the death of the child due to pulmonary failure

The prothrombin level fell to 40% in the cirrhotic patient and to 50% in the other icteric child but remained normal in the patient with anicteric hepatitis In those without cirrhosis all tests returned to normal and remained so for more than 2 years

Terminal cardiac liver

Three patients without cirrhosis died from re spiratory failure with cardiac livers during this study Apart from patient 36 with fatty infiltra tion the hepatic tests remained almost normal until death

DISCUSSION

In the 9 cirrhotic cases the clinical signs of an enlarged hard liver with or without splenomegaly preceded by several months the

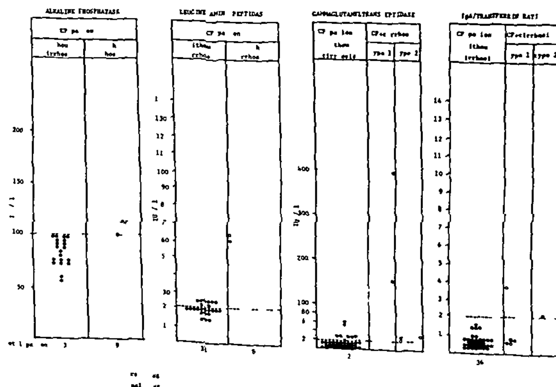


Fig 2 Cholestasis tests and IgA Transferrin ratio in 50 C F patients

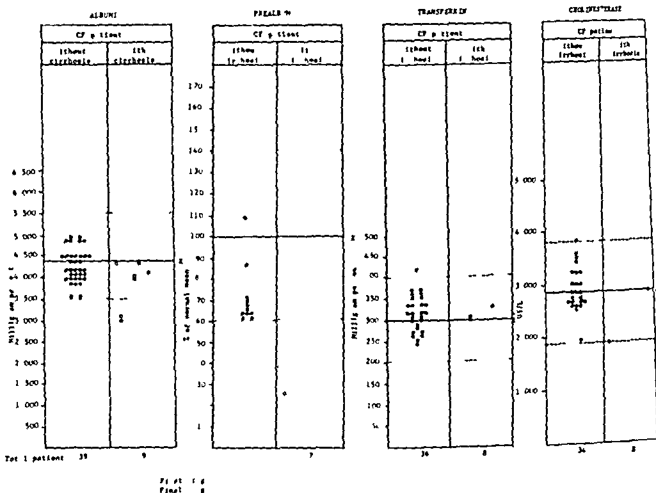


Fig 3 Hepatic cellular insufficiency tests in 50 C F patients

biochemical changes which therefore did not permit the early diagnosis of cirrhosis

Two distinct biochemical patterns emerged from the study First an obviously *cholestatic type* type 1 (Figs 1 2 3 and 4) with considerably abnormal cholestatic tests especially the γGT and significant cytolysis with raised OTC and B_{12} normal IgA and normal or high transferrin levels throughout giving an IgA Tr ratio(1 (patients 17 27 30 and 39) (Fig 2) Second a different biochemical type type 2 (nos 19 41 70 and 100) (Fig 2) where the cholestatic and cytolysis tests were only slightly affected The IgA levels however were consistently high with normal or low transferrin levels giving an IgA Tr ratio)1 9

These findings emphasised the importance of the IgA Tr ratio and its relationship to cholestasis and cytolysis One patient (no 17) however changed from type 1 to type 2

In the 41 patients without evidence of cirrhosis there was some inconsistency in the results of the various LFTs This long term observation showed that different hepatic functions were affected to a varying degree in each patient The isolated increase in OTC and B_{12} levels observed in patients whose lung function was satisfactory suggested a temporary or reduced degree of cytolysis with some degree of cholestasis Great variations in OTC levels may however be observed *Only a long term* study of non-C F children could allow a definite conclusion to be drawn

The disorders observed in this group were consistent with those found by other authors (7 15) who used slightly different tests or who measured the isoenzymes of alkaline phosphatase They confirmed the existence in about half the cases of CF of some hepatic disfunctions They may be an early indication

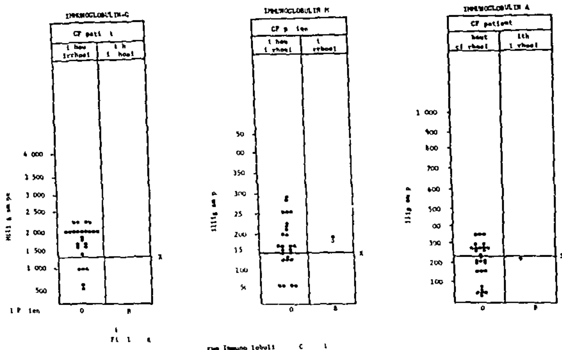


Fig 4 Serum immunoglobulins IgG IgM and IgA in 50 C F patients

of the development of multilobular cirrhosis but this could not be confirmed in the series over a period of 3 years On the other hand the return of tests to normal seems to indicate that the abnormalities are temporary and do not permanently affect hepatic function Hepatic disfunction therefore remains mainly latent in CF

The occurrence of viral hepatitis in 2 patients free from cirrhosis and the subsequent obser vation of them for 2 years showed that the hepatitis produced no further complications In particular no active chronic hepatitis nor short term evidence of cirrhosis occurred

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REFERENCES

1 Aron E Lamy J N & Weill J D *Le diagnostic* precoce de la cirrhose alcoolique du foie *Ann Gastroenterol Hepatol* 9 1 1973

2 Babson A L Greeley S J Coleman C N & Phillips G E The use of phenolphtaleine mono phosphate as substrate for S A P *Clin Chem* 12 8 1966

3 Benhamou J P Sicot Ch & Erlinger S Exposés de physiologie et de physiopathologie hépatique 1 Le débit sanguin hépatique *Presse Med* 79 185 1971

4 Berg T Serum levels of immunoglobulins in infancy and childhood A study of the normal development of IgG *IgA IgM IgD* and IgE and of the IgE levels in children with atopic diseases *Acta Univ Upsaliensis* 1969

5 Bodian M L *Fibrocystic disease of the pancreas* Heinemann London 1952 p 104

6 Craig J M Haddad H & Schwachman H The pathological changes in the liver in cystic fibrosis of the pancreas *Am J Dis Child* 93 357 1957

7 Dominick H Ch Hosemann R Van Husen N & *Gerlach U* About the clinical significance of the serum alkaline phosphatase isoenzymes in CF Comm 4th Annual Meeting European Working Group for Cystic Fibrosis Warsaw 4th Sept 1973

8 Ellman C Courtney D Andres V & Featherston R New and rapid colorimetric determination of acetyl cholinesterase activity *Biochem Pharmacol* 7 88 1961

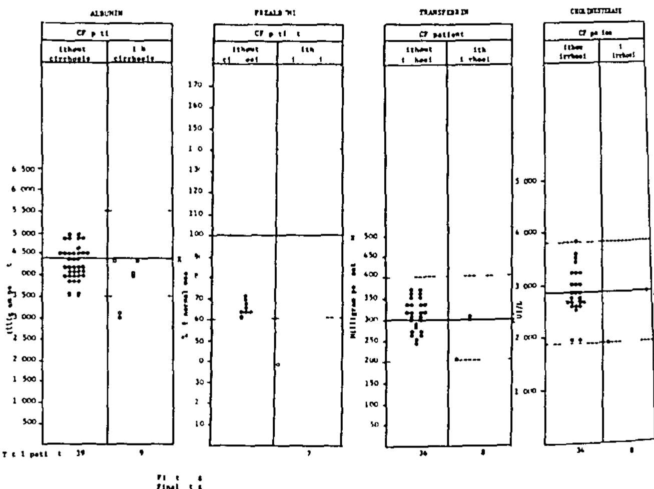


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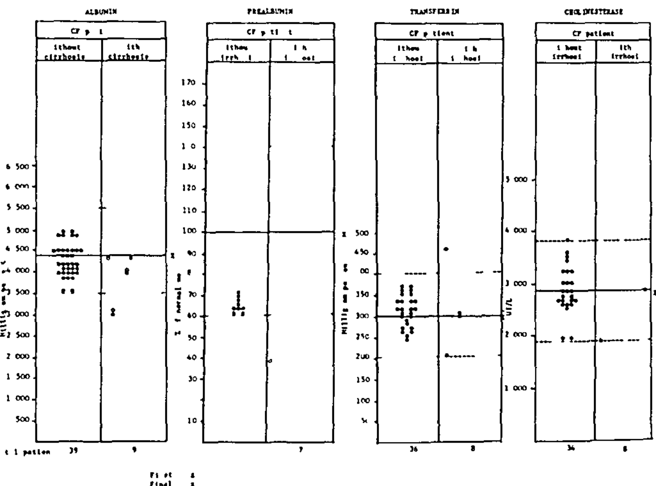


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Acta Pædiatr Scand 64 345–354 1975

RENAL BIOPSY STUDIES IN 150 CHILDREN WITH NON SPECIFIC GLOMERULOPATHY

K J VAN ACKER J VANDEN BRANDE and H HOLVOET

From the Departments of Paediatrics and Pathology State University Gent Belgium

ABSTRACT Van Acker K J Vanden Brande J and Holvoet H (Departments of Paediatrics and Pathology State University Gent Belgium) Renal biopsy studies in 150 children with non-specific glomerulopathy Acta Paediatr Scand 63 345 1974.—The experience with light microscopic examination of kidney biopsies in 150 children with non specific glomerulopathy is reported Most major types of glomerular lesions as they are at present known were observed but in a very uneven distribution Four groups of clinical symptoms were observed in the primary as well as in the associated non-specific glomerulopathies hematuria proteinuria acute nephritic syndrome and nephrotic syndrome Although in a number of instances, the histologic lesion was suggested by the clinical picture a correlation was lacking in most instances. More information was obtained from the histology when the evolution was considered particularly in the primary nephrotic syndrome and the Schonlein Henoch nephropathy

KEY WORDS Renal biopsy glomerulopathy children

From the histologic point of view two main classes of lesions can be distinguished in the glomerular nephropathies those lesions which are specific (amyloidosis thrombotic microangiopathy diabetic glomerulosclerosis) and the non specific lesions which do not allow a precise diagnosis of the underlying disease In this latter group the clinical features the morphologic aspects as shown by light and electron microscopy and by immunofluorescence and the correlation between the two have now been extensively studied

The purpose of the present work was to report the findings by light microscopy and the clinical symptoms in 150 children with non specific glomerulopathy

MATERIAL AND METHODS

All renal biopsies performed in this department since 196? were reviewed Nephropathies primarily due to diseases of the tubules or the interstitium and specific glomerulopathies (amyloidosis thrombotic microangiopathy) were then excluded from the study The remaining 217 biopsies concerned the non specific glomerulopathies They were obtained in 150 children from 8 months to 15 years old 2 or more biopsies being performed in 43 patients

With the exception of 8 surgical specimens all biopsies were obtained percutaneously Initially the Menghini needle was used but was soon replaced by the Silverman needle as modified by White (36) Although electron microscopy and immunofluorescence study were performed in several instances mainly during recent years these data were not taken into consideration in the present study Tissue fixation and staining were carried out according to generally accepted techniques The biopsies were examined by 3 independent investigators a classification of the lesions was used which was based on the published data of an international group of pathologists (11 15 20 38) For a detailed description of the different histologic types we refer to these authors The histologic data were then correlated with the clinical manifestations

RESULTS

Histologic findings

In general there was no substantial disagreement about the type of lesion observed in

9 Feigelson J Pecau Y & Sauvegrain J Liver function studies and biliary tract investigations in mucoviscidosis *Acta Paediatr Scand 59* 539 1970
10 Feigelson J Pecau Y & Perez R Liver scanning and liver function in cystic fibrosis *Acta Paediatr Scand 61* 337 1972
11 Feigelson J & Pecau Y L activité sérique de la gammaglutamyl transpeptidase dans les complications hépatiques de la mucoviscidose *Nouv Presse Med 1* 1899 1972
12 Gaudier B Nuyts J P Bombart F Debruxelles P & Ryckewaert Ph Les cirrhoses de la mucoviscidose *Lille Med 15* 1391 1970
13 Geiger H & Hoffmann P Quantitative immunologische Bestimmung von 16 verschiedenen Serumproteinen bei 260 normalen 0–15 Jahre alten Kindern *Z Kinderheilkd 109* 22 1970
14 Hallen J & Laurell C B Plasma Protein Pattern in cirrhosis of the liver *Scand J Clin Lab Invest 29* Suppl *124* 97 1972
15 Kattwinkel J Taussig L M Statland B E & Verter J I The effects of age on alkaline phosphatase and other serologic liver function tests in normal subjects and patients with cystic fibrosis *J Pediatr 83* 234 1973
16 Lelong M Joseph R Le Tan Vinh & Bouvattier Fibrose kystique du pancréas et hépatostéatose massive *Arch Fr Pediatr 7* 234 1950
17 Logrippo G A Anselm KI & Hayashi H Serum immunoglobulins and five serum proteins in extrahepatic obstructive jaundice and alcoholic cirrhosis *Am J Gastroenterol 54* 357 1971
18 Mollin D L & Anderson B Euglena method for assay of vitamin B_{12} *Personal communication* 1964
19 Nagel W Willig W & Schmidt F H Uber die aminosauere arylamidase aktivitat im menschlichen serum *Klin Wochenschr 42* 447 1964
20 Reitman S & Frankel S Colorimetric method for the determination of serum transaminase activity *Am J Clin Pathol 28* 1 1957
21 Ritland S Skrede S Blomhoff J P Gjone E Coagulation factors as indicators of protein synthesis in chronic liver disease *Scand J Gastroenterol 8* Suppl *19* 113 1973
22 di Sant Agnese P A & Blanc W A A distinctive type of biliary cirrhosis of the liver associated with cystic fibrosis of the pancreas *Pediatrics 18* 387 1956
23 Sitzmann F C & Stephan U Untersuchungen zur frage der leberbeteiligung bei der zystischen pankreasfibrose im kindesalter *Arch Kinderheilkd 175* 263 1967
24 Snodgrass Ph J & Parry D J The kinetics of serum ornithine carbamoyltransferase *J Lab Clin Med 73* 940 1969
25 Szasz G A kinetic photometric method for serum—glutamyl transpeptidase *Clin Chem 15* 124 1969
26 Turla B & Antonelli M Mucoviscidosi con steatosi epatica massiva *Riv Clin Pediatr 78* 485 1966
27 Tyson K R T Schuster S R & Shwachman H Portal hypertension in cystic fibrosis *J Pediatr Surg 3* 271 1968
28 Wilroy R S Crawford S E & Johnson W W Cystic fibrosis with extensive fat replacement of the liver *J Pediatr 68* 67 1966

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MATERIAL AND METHODS

All renal biopsies performed in this department since 1967 were reviewed Nephropathies primarily due to diseases of the tubules or the interstitium and specific glomerulopathies (amyloidosis thrombotic microangiopathy) were then excluded from the study The remaining 217 biopsies concerned the non specific glomerulopathies They were obtained in 150 children from 8 months to 15 years old 2 or more biopsies being performed in 43 patients

With the exception of 8 surgical specimens all biopsies were obtained percutaneously Initially the Menghini needle was used but was soon replaced by the Silverman needle as modified by White (36) Although electron microscopy and immunofluorescence study were performed in several instances mainly during recent years these data were not taken into consideration in the present study Tissue fixation and staining were carried out according to generally accepted techniques The biopsies were examined by 3 independent investigators a classification of the lesions was used which was based on the published data of an international group of pathologists (11 15 20 38) For a detailed description of the different histologic types we refer to these authors The histologic data were then correlated with the clinical manifestations

RESULTS

Histologic findings

In general there was no substantial disagreement about the type of lesion observed in

Table 1 *Distribution of lesions found on renal biopsy in the different clinical groups*
GP=Glomerulopathy GN=Glomerulonephritis

	Clinical picture								
	Hematuria		Proteinuria		Acute nephritic syndr		Nephrotic syndr		
Histologic type	Primary GP	Assoc GP	Primary GP	Assoc GP	Primary GP	Assoc GP	Primary GP	Assoc GP	Total (%)
1 Minimal lesions	29	5	10	–	4	3	49	1	101/(67 4)
2 Extramembranous GN	–	–	–	–	–	–	1	–	1/ (0 6)
3 Diffuse endocapill proliferative GN	–	–	1	–	4	–	3	–	8/ (5 3)
4 Membranoprolifer GN and lobular GN	2	–	–	–	–	–	11	–	13/ (8 7)
5 Diffuse endo-extracapill proliferative GN	–	2	1	–	1	3	3	–	10/ (6 7)
6 Segmental focal GN	2	1	–	–	1	–	4	2	10/ (6 7)
7 Segmental focal hyalinosis	–	–	–	–	–	–	2	–	?/ (1 3)
8 Global focal fibrosis	1	–	1	–	–	–	1	–	3/ (?)
9 Unclassifiable gl pathies (lesions too advanced)	–	–	–	–	2	–	–	–	2/ (1 3)
	34	8	13	–	12	6	74	3	

the different biopsy specimens Differences in opinion mainly concerned the minimal lesions and the discrete endocapillary proliferative lesions These biopsies were then reexamined until consent on the classification was obtained

The different lesions observed and the frequency with which they occurred in our patients are enumerated in Table 1 It appears that all the main types of non specific glomerular lesions as they are presently known are represented with the exception of mesangial sclerosis of which no examples were seen As could be expected the distribution of the different types is not uniform in a vast majority minimal lesions were diagnosed and some types are represented only once or a few times Repeat biopsy in 43 patients did not alter the histologic diagnosis in 2 patients with Schonlein Henoch syndrome regression of the extracapillary proliferation was observed

Clinical manifestations

The clinical symptoms and the biochemical abnormalities which have prompted us to perform the renal biopsy in these 150 patients can be classified into the following 4 groups according to the predominant symptom or syndrome macroscopic or microscopic hematuria proteinuria acute nephritic syndrome nephrotic syndrome In the patients with hematuria (which was accompanied by slight proteinuria in more than half of them) extensive investigations had permitted exclusion of gross abnormalities of the urinary tract urolithiasis urinary tract infection coagulation disorders and other known causes of hematuria No hearing loss was detected in these cases In the patients with proteinuria the latter had been found repeatedly during a period of at least 1 month protein excretion was usually moderate (less than 1 g/24 hours) but in one third of the patients it occasionally exceeded 3 g per 24 hours In exceptional cases the proteinuria was accompanied by microscopic hematuria IV urography was normal in all these patients and urine cultures remained sterile The acute nephritic syndrome was defined as the association of sudden macroscopic hematuria proteinuria oedema renal

ble 2 *Histologic lesions found on renal biopsy and evolution in 34 patients with hematuria*

ical presentation	Histologic lesion	Evolution
nosymptomatic hematuria 13 patients	Minimal lesions 12 patients	Cured 2 Persistent hematuria 7 Follow up<12 months 3
	Segmental focal GN 1 patient	Follow-up<12 months
ematuria+proteinuria 18 patients	Minimal lesions 16 patients	Cured 1 Persistent hematuria 8 Follow up<1 months 7
	Segmental focal GN 1 patient	Persistent hematuria
	Global focal fibrosis 1 patient	Persistent hematuria
Iematuria+proteinuria+hypertension 3 patients	Membranoprolifer GN 2 patients	Died 1 Slight hypert traces proteinuria 1
	Minimal lesions 1 patient	Follow up<12 months

insufficiency and eventually hypertension The nephrotic syndrome was defined as the association of hypoalbuminemia (less than 2 5 g/100 ml) pronounced proteinuria (more than 100 mg/kg/24 hours) and oedema occasionally accompanied by hematuria hypertension or renal insufficiency

On clinical grounds it was obvious that in some patients the glomerulopathy was part of a generalized disease either well defined (purpura Schonlein Henoch) or not (systemic like disease with such symptoms as arthritis rash thrombocytopenic purpura persistent fever but no biologic proof of systemic lupus erythematosus) In these 17 patients the nephropathy was called associated glomerulopathy In the great majority however (133 patients) the glomerulopathy apparently occurred as an isolated disease it was therefore called primary glomerulopathy

In some patients from both groups a progression from one clinical type to another was observed these patients were classified according to the most prominent clinical feature

Clinical pathological correlation in the primary glomerulopathies

Thirty four patients 21 boys and 13 girls underwent renal biopsy because of either persistent microscopic *hematuria* (11 patients) or one or more attacks of macroscopic hematuria usually alternating with microscopic hematuria (23 patients) (Table 2) Preceding infection of the upper respiratory tract was mentioned in 26 of the 34 patients (76%) ASLO titres were determined in 28 patients a titre of more than 250 U was found in 8 Most patients however were examined only several months after detection of the hematuria C_3 fraction of serum complement (β_1C) was measured in 19 patients and was decreased in only one with membrano proliferative GN [1] In 7 patients microscopic hematuria was also observed in a family member but examination of the urine sediment was not performed in the family members of all patients One patient with membrano-proliferative GN had a sibling with the same histologic lesion but with

[1] GN=glomerulonephritis

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Histologic type	Clinical picture: Hematuria, Primary GP	Hematuria, Assoc GP	Proteinuria, Primary GP	Proteinuria, Assoc GP	Acute nephritic syndr, Primary GP	Acute nephritic syndr, Assoc GP	Nephrotic syndr, Primary GP	Nephrotic syndr, Assoc GP	Total (%)
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5 Diffuse endo-extracapill proliferative GN	–	2	1	–	1	3	3	–	10/ (6 7)
6 Segmental focal GN	2	1	–	–	1	–	4	2	10/ (6 7)
7 Segmental focal hyalinosis	–	–	–	–	–	–	2	–	2/ (1 3)
8 Global focal fibrosis	1	–	1	–	–	–	1	–	3/ (2)
9 Unclassifiable gl pathies (lesions too advanced)	–	–	–	–	2	–	–	–	2/ (1 3)
	34	8	13	–	12	6	74	3	

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Table 4 Histologic lesions found on renal biopsy and evolution in 12 patients with acute nephritic syndrome

Clinical presentation	Histologic lesion	Evolution
	Minimal lesions 4 patients	Cured 1 Residual hematuria 1 Residual hemat +proteinuria 2
	Diffuse endocapillary prolifer GN *4 patients*	Cured 2 Residual symptoms 2
Acute nephritic syndrome	Segmental focal GN 1 patient	Residual hematuria
	Diffuse endo-extracapillary prolifer GN *1 patient*	Died (rapidly progressive renal insuffic)
	Unclassifiable lesions 2 patients	Died 1 (rapidly progressive renal insuffic) Chronic hemodialysis 1

respiratory tract had been noted in 7 patients Microscopic hematuria was present in 2 microscopic hematuria and transient hypertension was observed in 1 and deficient concentrating capacity in another one With the exception of the latter all patients had normal renal functions ASLO titres and serum β_1C levels were determined only occasionally 2 patients had a raised ASLO titre (≥250 U) in the 3 patients in whom it was determined β_1C was normal The biopsies were performed 1 month to more than 9 years after the detection of the proteinuria (mean 36 months) In 10 of the 13 patients including the one with transient hypertension only minimal lesions were found During a follow up period varying from 2 months to practically 7 years the proteinuria disappeared in 2 and persisted in 8 No deterioration of the renal function was observed In one patient diffuse endocapillary proliferation was present Follow up in this patient was insufficient proteinuria was still present after 2 months In a second patient the biopsy revealed diffuse endo- end extracapillary proliferative GN Eleven years previously this patient had been admitted for a nephrotic syndrome Without treatment the symptoms had disappeared in the course of a few months but proteinuria and after 9 years also microscopic hematuria was observed regularly A third patient had global focal fibrosis When first seen there was no associated hematuria and blood pressure was normal but there was a deficient renal concentrating capacity The situation remained unchanged after 2½ years

Twelve patients 6 girls and 6 boys with a mean age of 9 years 6 months presented with an *acute nephritic syndrome* as defined earlier (Table 4) Preceding infection of the upper respiratory tract was mentioned in all but one In 10 of the 11 patients in whom it was determined ASLO titre was elevated (≥250 U) Throat cultures were taken in 9 patients but grew β hemolytic streptococci in only one most patients however had been treated with antibiotics before admission and some were seen some time after the onset of the symptoms Serum β_1C levels were determined in only 3 patients in 1 a rise from low to normal values was observed and in the other 2 β_1C was normal

In 4 patients the biopsy revealed minimal lesions the biopsy was performed 16 days to 6 months after the appearance of the first symptoms The follow up period in the 4 patients varied from 1½ months to 11 months clinical symptoms had then disappeared in all of them One patient had no urinary ab

Table 3 *Histologic lesions found on renal biopsy and evolution in 13 patients with proteinuria*

Clinical presentation	Histologic lesion	Evolution
Monosymptomatic proteinuria 9 patients	Minimal lesions 7	Cured 2 Persistent proteinuria 5
	Diffuse endocapillary prolifer GN 1	Follow up<12 months
	Diffuse endo-extracapillary prolifer GN 1	Persistent proteinuria 2
Proteinuria+microsc hematuria 2 patients	Minimal lesions 2	Persistent proteinuria 2
Proteinuria+microsc hematuria+hypertension 1 patient	Minimal lesions	Persistent proteinuria
Proteinuria+deficient concentrat capacity 1 patient	Global focal fibrosis	Persistent proteinuria+ defic concentr capac

a different clinical picture these patients have been described elsewhere (35)

In the vast majority i e in 31 of the 34 patients the hematuria accompanied in 18 cases by a slight mostly inconstant proteinuria was the only abnormality renal function was normal and there was no hypertension The biopsies in these patients were performed 1 month to 7 years after the detection of the hematuria (mean 22 months) Light microscopic examination revealed minimal lesions in all but 3 in 1 global focal fibrosis and in 2 segmental focal GN was found

The duration of the follow up period in these 31 patients was variable In 11 it was less than 12 months and therefore considered as insufficient all these patients still had hematuria at the last examination One patient with segmental focal GN belonged to this group In the remaining 20 patients the follow up period varied from 18 to 91 months (mean 43 months) The hematuria completely disappeared in 3 hematuria was still present at the last examination in 17 No deterioration of the renal function was observed in any of the 20 patients including the second patient with segmental focal GN (follow up 91 months)

The 3 other patients with hematuria also had proteinuria but in addition arterial hypertension was noted In 1 there was a severe hypertension after treatment with corticosteroids for 2 months renal function rapidly deteriorated and death occurred after 8 months Low serum β_1C levels and normal ASLO titres were observed in this patient Renal biopsy revealed membrano-proliferative GN

Hypertension in the remaining 2 patients was much less marked renal functions were normal In one the biopsy revealed membrano proliferative GN serum β_1C levels were normal ASLO titres were elevated on one occasion The patient was followed up for 6 years no abnormalities were seen at the last examination except for traces of protein in the urine and slight hypertension The second patient had transient hypertension when the biopsy was performed 10 years after the detection of the hematuria Microscopic examination revealed minimal lesions The patient was lost for follow up

In 13 patients 9 girls and 4 boys the renal biopsy was performed because of *proteinuria* (Table 3) In most cases the proteinuria was detected at routine urinalysis only in 5 was oedema of the eyelids mentioned but a nephrotic syndrome never developed Repeated infections of the upper

Table 5 *Sex ratio mean age at onset histologic lesions found on renal biopsy response to corticosteroid treatment and evolution in 74 patients with nephrotic syndrome*

Clinical presentation	Male/female ratio	Mean age at onset	Histologic lesion	Response to steroid treatment	Evolution
Pure nephrotic syndrome 48 patients	3 8 1	67 months	Minimal lesions 47	Not treated 2	Remission no relapses 2
				Good resp 45	Remission 45 Frequent relapses 30
			Diffuse endocap prolif GN 1	Good resp	Remission Frequent relapses
Mixed nephrotic syndrome 6 patients	0 8 1	81 months	Minimal lesions ?	Resistant 2	Remission 1 Died 1
			Extramembran GN 1	Second resistant	Proteinuria
			Diffuse endocap prolif GN 2	Good resp 2	Remission 1 Died 1 (thrombosis)
			Membrano-prolif GN 11	Not treated 2	Remission 1 Died 1
				Resistant 9	Remission 1 Nephrotic syndr 4 Renal insuff 1 Died 3
			Diffuse endo-extracap prolif GN 3	Not treated 1 Resistant ?	Died Proteinuria 1 Died 1
			Segmental focal GN 4	Resistant 4	Remission 1 Died 3
			Segmental focal hyalinosis 2	Resistant ?	Remission 1 Died 1
			Global focal fibrosis 1	Resistant	*Renal insuff*

Three patients were never treated with corticosteroids The remaining 23 did receive such treatment initially for months or even years but more recently only for 8 weeks if no response was seen Cytotoxic drugs were then used in the latter cases Good response to corticosteroid treatment was exceptional it occurred in only 2 cases with diffuse endocapillary proliferative GN In the remaining 21 patients the steroid treatment had no appreciable effect 1 with extramembranous GN became resistant during the first relapse

The evolution in these patients varied widely (Table 5) On the whole 6 patients went into remission in 6 marked proteinuria or a nephrotic syndrome persisted 2 had renal insufficiency and 12 died The duration of the follow up period in the living patients varies from 5 months to more than 12 years (mean 57 months) It should be noted that in the patients with membrano proliferative GN and low β_1C levels the latter did not rise even when the clinical syndrome disappeared

Clinical pathological correlation in associated glomerulopathies (Table 6)

In the 3 patients with systemic like disease the clinical symptoms of the nephropathy consisted of renal insufficiency macroscopic or microscopic hematuria and proteinuria Pronounced arterial hypertension was present in 1 and transient oedema in 2 patients Repeated investigations for lupus erythematosus remained negative Renal biopsy which was repeated in 2 patients revealed no abnormalities

normalities in the other 3 there was a residual microscopic hematuria accompanied by proteinuria in 2 but normal renal functions

In 4 other patients the biopsy performed between 12 days and 2 months after the appearance of the first symptoms showed diffuse endocapillary proliferation The patients were followed for 1/2 month to 4 years 7 months Two of them were completely healed in 2 with a follow up period of 1/2 and 1 month microscopic hematuria slight proteinuria and a decreased glomerular filtration rate were still present

In 1 patient the biopsy performed after 13 months revealed segmental focal GN ASLO titre was normal at that time Microscopic hematuria was the only abnormal finding after 4 years In 1 patient the biopsy performed 7 months after the appearance of the symptoms revealed diffuse endo- and extracapillary proliferation The acute nephritic syndrome rapidly progressed towards severe renal insufficiency with microscopic hematuria proteinuria and hypertension Death occurred after 7 months A closely comparable evolution was seen in another patient with unclassifiable lesions here death occurred after an evolution of 1 year In both patients ASLO titres were elevated at the onset of the disease

In a last patient the evolution was much slower The disease started as a typical acute nephritic syndrome which subsided slowly Multiple attacks of macroscopic hematuria then occurred in the following years and progressive renal insufficiency developed The biopsy was performed 10 years after the appearance of the first symptomes the lesions were too advanced to be classified

In the last group of 74 patients the biopsy was performed because of a *nephrotic syndrome* (Table 5) On clinical grounds a further distinction was made between pure nephrotic syndromes in which hematuria renal insufficiency and (or) hypertension were not present at onset or eventually developed, but disappeared within the first month of evolution and the mixed nephrotic syndromes in which one or more of these symptoms persisted beyond the first month Selectivity of proteinuria was studied only in the more recent patients and is therefore not taken into consideration

Forty eight patients belonged to the pure nephrotic syndrome group There were 38 boys and 10 girls (ratio 3 8 1) the mean age at onset was 67 months Serum β_1C levels were determined in 32 patients and were always within normal limits Renal biopsy revealed minimal lesions in all but one in whom diffuse endocapillary proliferative GN was observed

Two patients of the 48 did not receive corticosteroid treatment complete recovery occurred within 1 month in both and relapses were never seen during a follow up period of 8 and 47 months respectively The remaining 46 patients including the one with endocapillary proliferative GN were treated with corticosteroids as described elsewhere (33) A good response resulting in the disappearance of the oedema proteinuria and serologic abnormalities was observed in all of them During follow up periods ranging from 7 months to more than 14 years (mean 4 years 8 months) death or evolution towards renal insufficiency was never observed Relapses however frequently occurred in 30 patients (mean 3 5 per patient) Treatment with corticosteroids again resulted in remission in all patients in some with multiple relapses cytotoxic drugs were used later

Twenty six patients belonged to the mixed nephrotic syndrome group There were 12 boys and 14 girls (ratio 0 8 1) the mean age at onset was 81 months Serum β_1C levels were determined in 11 patients they were normal in 6 (2 of them with membrano-proliferative GN) and persistently low in 5 all with membrano proliferative GN Renal biopsy revealed minimal lesions in 2 patients in the remaining 24 the different histologic types enumerated in Table 5 were observed

revealed lesions which were not apparent on light microscopy This has been demonstrated in the literature (3 29) and also appears from our more recent investigations

In agreement with what has been found in larger series (7 8 11 20 26 38 39) there is no clearcut correlation in our patients with primary glomerulopathies between the presenting clinical picture and the type of underlying glomerular lesion the same lesions are observed in different clinical groups and identical symptoms are seen with different lesions At most the type of glomerular lesion is suggested by the clinical symptoms in a few instances Thus a pure nephrotic syndrome in a young child who had a good response to corticosteroid treatment was mostly associated with minimal lesions Persistent hypocomplementemia was invariably seen in patients with membranoproliferative GN but the reverse was not necessarily true Rapidly progressing renal insufficiency in a child with acute nephritic syndrome was associated with extra and endocapillary proliferative lesions These findings are also in good agreement with the literature (6 15 16 20 22 39)

The data provided by renal biopsy have above all a prognostic value This is particularly evident in our patients with primary nephrotic syndrome and those with Schonlein Henoch nephropathy The correlation is less apparent in the other groups probably because of the insufficient follow up of some patients and the lack of complementary examinations like the immunofluorescence study in others The literature data clearly show that within certain limits the evolution in the glomerulopathies can be correlated with the underlying glomerular lesion irrespective of the clinical picture This has been demonstrated in large series of patients with minimal lesions extramembranous GN focal glomerulosclerosis membranoproliferative GN and mesangial sclerosis it is noteworthy that not all pronounced lesions need be fatal (6 9 13 17 19 20 22 35 39) The prognostic significance of other lesions e g the diffuse endocapillary proliferation is less well defined It is obvious that these statistical figures do not always permit firm prognostic conclusions in each individual case In the associated glomerulopathies above all the Schonlein Henoch syndrome a correlation between the type of glomerular lesion the degree of extension of some lesions and the evolution has been demonstrated (18 24 28) The present material is too small to allow any conclusions in this respect

REFERENCES

1 Abramowicz M Arneil G C Barnett H L Barron B A Edelman C M Gordillo-P G Greifer I Hallman N Kobayashi O & Tiddens H A Controlled trial of azathioprine in children with nephrotic syndrome *Lancet I* 959 1970

2 Arneil G C 164 children with nephrosis *Lancet II* 1103 1961

3 Berger J IgA glomerular deposits in renal disease *Transplant Proc I* 939 1969

4 Brodehl J Steroide oder Zytostatika beim kindlichen nephrotischen Syndrom *Klin Paediatr 184* 241 1972

5 — Immunosuppressive Behandlung des nephrotischen Syndroms im Kindesalter *Monatsschr Kinderheilk 120* 273 1972

6 Cameron J S Glasgow E F Ogg C S & White R H R Membranoproliferative glomerulonephritis and persistent hypocomplementaemia *Br Med J IV* 7 1970

7 Cameron J S Glomerulonephritis *Br Med J IV I* 285 1970

8 — Nephrotic Syndrome *B Med J IV* 350 1970

9 Cameron J S Ogg C S Turner D R & Weller R O *Membranous nephropathy* Communication at *the 6th ann meeting of the Europ Soc Ped Nephrol* Dublin 1972

10 Cameron J S *Bright s disease today the pathogenesis* and treatment of glomerulonephritis Part I *Br Med J IV* 87 1972 Part II ibid *IV* 160 1972 Part III ibid IV 217 1972

11 Churg J Habib R & White R H R Pathology of the nephrotic syndrome in children *Lancet I* 1299 1970

12 Dodge W F Spargo B H Travis L B Srivastava R N Carvajal H F De Beukelaer M M Longley M P & Menchaca J A Poststreptococcal glomerulonephritis *N Engl J Med 286* 273 197

13 Focal glomerulosclerosis *Lancet II* 367 197

14 Glasgow E F Moncrieff M W & White R H R Symptomless haematuria in childhood *Br Med J II* 687 1970

15 Habib R *Classification anatomique des nephro-*

Table 6 *Clinical picture histologic lesions found on renal biopsy and evolution in 17 patients with associated glomerulopathy*

	Clinical picture	Histologic lesion	Evolution
Systemic like disease 3 patients	Renal insufficiency+hematuria+ proteinuria 2 patients id +hypertension 1 patient	Minimal lesions 3	Died 2 Cured 1
Schonlein Henoch syndrome 14 patients	Hematuria 8 patients	Minimal lesions 5	Cured 4 Microsc hemat 1
		Diff endo-extracap prolifer GN 2	Hematuria 1 Proteinuria 1
		Segmental focal GN 1	Cured 1
	Acute nephritic s 3 patients	Diff endo-extracap prolifer GN 3	Cured 1 Died 1 Hypertension 1
	Nephrotic syndr 3 patients	Minimal lesions 1	Cured 1
		Segmental focal GN 2	Cured 1

All patients were treated with corticosteroids cytotoxic drugs and 1 also received antimalarial drugs Two patients died after respectively 2 and 4 years in the third patient no clinical or biochemical abnormalities are observed after more than 2 years

In 14 patients the nephropathy was associated with classical Schonlein Henoch syndrome with rash non thrombocytopenic purpura arthralgia and (or) abdominal pain The symptoms were the same as in the primary glomerulopathies hematuria acute nephritic syndrome and nephrotic syndrome with microscopic or macroscopic hematuria The biopsies revealed 3 histologic patterns minimal lesions diffuse endo and extracapillary proliferative GN and segmental focal GN

The small number of patients does not permit any correlation of the clinical picture with the underlying histologic lesion it can be noted however, that the 3 types of lesions were found in patients with hematuria and that 1 patient with nephrotic syndrome had minimal lesions

As far as the outcome is concerned some correlation with the histologic lesions can be seen all patients with minimal lesions healed or showed only microscopic hematuria and the 3 patients with segmental focal GN became cured Of the 5 patients however with endo and extracapillary proliferative GN 1 died 3 had sequelae (hypertension proteinuria hematuria) and only 1 was cured

DISCUSSION

The present retrospective study represents the experience with renal biopsy of a University Paediatric Department with interest in nephrologic problems in children over a period of 10 years Approximately 4/5 of these patients were referred from different parts of the country therefore the population from which they are drawn cannot be calculated The fact that the department acts mainly as a referral centre should be taken into account when the relative frequency of the different histologic types is considered

Judging from the experience from other centres it is probable that the correct histologic diagnosis has been missed in a number of our cases This is to be expected in for example segmental focal glomerulosclerosis where the lesions are found predominantly around the corticomedullary junction and therefore will be easily missed with a superficial needle biopsy Furthermore it cannot be denied that in some patients systematic fluorescence studies would probably have

revealed lesions which were not apparent on light microscopy This has been demonstrated in the literature (3 29) and also appears from our more recent investigations

In agreement with what has been found in larger series (7 8 11 20 26 38 39) there is no clearcut correlation in our patients with primary glomerulopathies between the presenting clinical picture and the type of underlying glomerular lesion the same lesions are observed in different clinical groups and identical symptoms are seen with different lesions At most the type of glomerular lesion is suggested by the clinical symptoms in a few instances Thus a pure nephrotic syndrome in a young child who had a good response to corticosteroid treatment was mostly associated with minimal lesions Persistent hypocomplementemia was invariably seen in patients with membranoproliferative GN but the reverse was not necessarily true Rapidly progressing renal insufficiency in a child with acute nephritic syndrome was associated with extra and endocapillary proliferative lesions These findings are also in good agreement with the literature (6 15 16 20 22 39)

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REFERENCES

1 Abramowicz M Arneil G C Barnett H L Barron B A Edelman C M Gordillo-P G Greifer I Hallman N Kobayashi O & Tiddens H A Controlled trial of azathioprine in children with nephrotic syndrome *Lancet I* 959 1970

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4 Brodehl J Steroide oder Zytostatika beim kindlichen nephrotischen Syndrom *Klin Paediatr 184* 241 1972

5 — Immunosuppressive Behandlung des nephrotischen Syndroms im Kindesalter *Monatsschr Kinderheilk 120* 273 1972

6 Cameron J S Glasgow E F Ogg C S & White R H R Membranoproliferative glomerulonephritis and persistent hypocomplementaemia *Br Med J IV* 7 1970

7 Cameron J S Glomerulonephritis *Br Med J IV I* 285 1970

8 — Nephrotic Syndrome *Br Med J IV* 350 1970

9 Cameron J S Ogg C S Turner D R & Weller R O *Membranous nephropathy Communication at the 6th ann meeting of the Europ Soc Ped Nephrol* Dublin 1972

10 Cameron J S Bright s disease today the pathogenesis and treatment of glomerulonephritis Part I *Br Med J IV* 87 1972 Part II ibid *IV* 160 1972 Part III ibid IV 217 1972

11 Churg J Habib R & White R H R Pathology of the nephrotic syndrome in children *Lancet I* 1299 1970

12 Dodge W F Spargo B H Travis L B Srivastava R N Carvajal H F De Beukelaer M M Longley M P & Menchaca J A Poststreptococcal glomerulonephritis *N Engl J Med 286* 273 1972

13 Focal glomerulosclerosis *Lancet II* 367 1972

14 Glasgow E F Moncrieff M W & White R H R Symptomless haematuria in childhood *B Med J II* 687 1970

15 Habib R *Classification anatomique des nephro-*

pathies glomerulaires Padiat Fortbildungskurse fur die Praxis E Rossi (ed) Karger Basel 1970
16 Habib R Kleinknecht Cl & Royer P Le syndrome néphrotique primitif de l enfant *Arch Fr Pediatr 28* 277 1971
17 Habib R & Gubler M C Les lésions glomérulaires focales des syndromes néphrotiques idiopathiques de l enfant *Nephron 8* 382 1971
18 Habib R & Levy M Les néphropathies du purpura rhumatoide chez l enfant *Arch Fr Pediatr 29* 305 1972
19 Habib R Extramembranous glomerulonephritis in children report of 50 cases *J Pediat 82* 754 1973
20 — Classification of glomerulonephritis based on morphology P Kincaid Smith T H Mathew & E L Becker (ed) *Proc Intern Symp* John Wiley New York 1973
21 Hendler E D Kashgarian M & Hayslett J P Clinicopathological correlations of primary haematuria *Lancet I* 458 1972
22 Holland N H & Bennet N M Hypocomplementemic (membranoproliferative) glomerulonephritis *Am J Dis Child 123* 439 1972
23 Hoyer J R Raij L Vernier R L Simmons R L Najarian J S & Michael A F Recurrence of idiopathic nephrotic syndrome after renal transplantation *Lancet II* 343 1972
24 Hurley R M & Drummond K N Anaphylactoid purpura nephritis clinicopathological correlations *J Pediatr 81* 904 1972
25 Hutt M S R & White R H R A clinico pathological study of acute glomerulonephritis in east african children *Arch Dis Child 39* 313 1964
26 Kuyten R Nierbiopsie bij kinderen met glomerulopathieen *Proefschrift* Amsterdam 1969
27 McConville J M West C D & McAdams A J Familial and nonfamilial benign hematuria *J Pediatr 69* 207 1966
28 Meadow S R Glasgow E F White R H R Moncrieff M W Cameron J S & Ogg C S Schonlein Henoch nephritis *Quart J Med 41* 241 1972
29 Morel Maroger L Leathem A & Richet G Glomerular abnormalities in nonsystemic diseases *Am J Med 53* 170 1972
30 The nephritis of Schonlein Henoch syndrome *Lancet II* 413 1972
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Table 1 *Pertinent data on the material*

Diabetics					Controls		
Number of cases	Sex	Age at examination (yrs ±S D)	Duration of diabetes (yrs ±S D)	Insulin dose (I U)	Number of cases	Sex	Age at examination (yrs ±S D)
15	M	11 0±3 6	4 9±3 1	33±15	29	M	10 1±2 1
17	F	11 5±2 8	4 5±2 1	40±18	20	F	8 9±1 8

MATERIAL AND METHODS

Thirty two diabetic children were investigated at their regular visit to the outpatient clinic and 49 healthy children chosen from a youth centre near the hospital were similarly studied (pertinent data see Table 1) All the diabetic children received insulin usually small amounts of crystalline mixed with NPH insulin None of the diabetic children had signs of retinopathy or nephropathy At the time of the examination none were acidotic (i e arterial pH>7 37 standard bicarbonate>22 mmol/l) but their blood glucose values varied greatly The control children were selected according to age and sex in order to compare them with the diabetic children None of the children were smokers and none had anemia (haemoglobin>12 g/100 ml)

Capillary and venous blood samples were taken in the morning following 15 minutes of complete rest A light breakfast and the usual morning insulin dose were permitted The capillary blood was analysed for pH blood gas measurements and for glucose using an autoanalyzer glucose oxydase method Of the venous blood sample 3 ml stabilized with heparin was immediately placed on ice and within *10 min 1 ml* was added to *5 0 ml* chilled *0 6 M* perchloric acid The blood extracts were frozen at −20°C until analysed for 2 3 DPG according to the method of Ericsson & de Verdier (15) One sample was centrifuged at 5000 *g* for 10 min and the plasma removed for inorganic phosphate determination (25) Another sample was used for measurements of haemoglobin (21) haematocrit red cell count and red cell indices by means of a Coulter S counter A 10 ml heparinized sample was used within 30 min for the determination of the dissociation curve employing the dissociation curve analyzer (type DCA 1 Radiometer Copenhagen) providing a continuous print out of the ODC of whole blood (14) From the recorded curves P_{25} P_{50} and P_{75} (oxygen tension at 25% 50% and 75% saturation) at pH of 7 40 and the *in vivo* pH were calculated from the formula log P=log T+0 38 (pH 7 40) where T is the Po_2 corresponding to respective saturations (16 28)

Rank sum tests correlation and multiple regression analyses were used for statistical analyses [1]

RESULTS

No distinction was made for sex and age in the figures quoted since no significant differences were found

Haematological parameters Haemoglobin concentration haematocrit erythrocyte count mean corpuscular volume and mean corpuscular haemoglobin were significantly larger while the MCHC was lower in the diabetic than in the control children The average 2 3 DPG content of the erythrocytes was significantly increased among the diabetic children (Table 2)

2 3 DPG and haemoglobin concentration Fig 1 shows that there was a significant inverse relationship between the 2 3 DPG content and

[1] The statistical analyses were performed in collaboration with lic ing P Thoft Christensen Aalborg

Table 2 *Average haemoglobin erythrocyte count haematocrit mean corpuscular haemoglobin mean corpuscular volume mean corpuscular haemoglobin concentration leucocyte count and 2 3 diphosphoglycerate content (±S D) in diabetic and healthy children*

Groups	Haemoglob (g/100 ml)	Erythrocyt (×10 [12]/l)	Haematocrit (volfr)	MCH (fmol)	MCV (fl)	MCHC (mmol/l)
Diabetic children	13 7±0 82	4 8±0 28	0 42±0 03	1 78±0 079	87 9±3 35	20 0±0 71
Healthy children	13 1±0 44	4 6±0 14	0 40±0 02	1 75±0 063	85 3±3 14	20 4±0 68
Significance	$p<0\ 001$	$p<0\ 02$	$p<0\ 001$	$p<0\ 05$	$p<0\ 005$	$p<0\ 05$

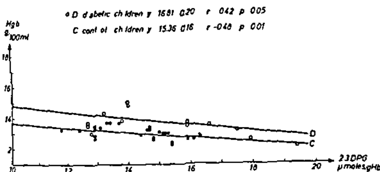


Fig 1 The correlation between 2,3 diphosphoglycerate content and haemoglobin concentration in diabetic and in healthy children

the haemoglobin concentration both among the diabetics ($r=-0\,42$ $p<0\,05$) and among the healthy children ($r=-0\,48$ $p<0\,01$) There is no significant difference between the slopes of the two regression lines which means that at all haemoglobin levels an additional increase in 2 3 DPG was present in the children with diabetes Among the diabetic children there was found a significant inverse relationship between the 2 3 DPG and the MCHC ($r=-0\,43$ $p<0\,02$) but this relationship was not present among the healthy children No relationship was found between 2 3 DPG and the haematocrit values or between 2 3 DPG and the duration of diabetes

2,3 DPG and blood sugar Blood sugar concentrations averaged 12 55±6 21 mmol/l (mean ±S D) among the diabetic children and 5 04±0 97 mmol/l among the healthy children ($p<0\,001$) Neither among the diabetic nor among the healthy children were there any correlation between the 2 3 DPG and the blood sugar level

Leucocytes (×10⁹/l)	2 3 DPG	
	mmol/l RBC	µmol/g Hb
6 01±1 55	4 96±0 5?	15 ?±1 70
6 48±1 6?	4 5?±0 40	13 9±1 31
n s	$p<0\,001$	$p<0\,00?$

n s =non significant

2 3 DPG and plasma inorganic phosphate (P_i) P_i among 23 diabetic children averaged 1 51±0 22 mmol/l (mean ±S D) which was significantly higher than the average of 1 37±0 23 mmol/l among 19 healthy children ($p<0\,05$) There was a slightly positive correlation between the P_i and the 2 3 DPG concentration when all children are considered collectively ($r=0\,32$ $p<0\,05$)

2 3 DPG and P_{50} There was a positive correlation between the 2 3 DPG content and the P_{50} (*in vivo* pH) among the diabetic children ($r=0\,66$ $p<0\,01$) but not so for the healthy children (Fig 2) Similarly there was a positive correlation between the 2 3 DPG content and the P_{50} (7 40) of the ODC among the diabetic children ($r=0\,66$ $p<0\,01$) whereas this correlation did not reach a level of significance among the healthy children ($r=0\,19$)

Despite the average increase in 2 3 DPG the P_{50} was not increased in the diabetic children (Fig 3) The average P_{50} (7 40) was 26 32±1 40 mmHg (mean ±S D) among the diabetic children and 26 40±0 98 mmHg among the healthy children ($t=0\,11$) The average P_{50} (*in vivo* pH) was 25 37±1 37 mmHg (mean ±S D) in the diabetic children as opposed to 25 11±0 94 mmHg in the healthy children ($t=-0\,91$)

P_{50} and MCHC There was a strong inverse correlation between the MCHC and the P_{50} (*in vivo* pH) ($r=-0\,59$ $p<0\,01$) while such a relationship was not present among the control children ($r=-0\,05$) A similar inverse relationship was also present in the diabetic children

Table 1 *Pertinent data on the material*

Diabetics					Controls		
Number of cases	Sex	Age at examination (yrs ±S D)	Duration of diabetes (yrs ±S D)	Insulin dose (I U)	Number of cases	Sex	Age at examination (yrs ±S D)
15	M	11 0±3 6	4 9±3 1	33±15	29	M	10 1±2 1
17	F	11 5±2 8	4 5±2 1	40±18	20	F	8 9±1 8

MATERIAL AND METHODS

Thirty two diabetic children were investigated at their regular visit to the outpatient clinic and 49 healthy children chosen from a youth centre near the hospital were similarly studied (pertinent data see Table 1) All the diabetic children received insulin usually small amounts of crystalline mixed with NPH insulin None of the diabetic children had signs of retinopathy or nephropathy At the time of the examination none were acidotic (i e arterial pH>7 37 standard bicarbonate>22 mmol/l) but their blood glucose values varied greatly The control children were selected according to age and sex in order to compare them with the diabetic children None of the children were smokers and none had anemia (haemoglobin>12 g/100 ml)

Capillary and venous blood samples were taken in the morning following 15 minutes of complete rest A light breakfast and the usual morning insulin dose were permitted The capillary blood was analysed for pH blood gas measurements and for glucose using an autoanalyzer glucose oxydase method Of the venous blood sample 3 ml stabilized with heparin was immediately placed on ice and within 10 min 1 ml was added to 5 0 ml chilled 0 6 M perchloric acid The blood extracts were frozen at −20°C *until analysed for 2 3 DPG according to the method of* Ericsson & de Verdier (15) One sample was centrifuged at 5 000 g for 10 min and the plasma removed for inorganic phosphate determination (25) Another sample was used for measurements of haemoglobin (21) haematocrit red cell count and red cell indices by means of a Coulter S counter A 10 ml heparinized sample was used within 30 min for the determination of the dissociation curve employing the dissociation curve analyzer (type DCA 1 Radiometer Copenhagen) providing a continuous print out of the ODC of whole blood (14) From the recorded curves P_{25} P_{50} and P_{75} (oxygen tension at 25% 50% and 75% saturation) at pH of 7 40 and the *in vivo* pH were calculated from the formula log P=log T+0 38 (pH 7 40) where T is the P_{O_2} corresponding to respective saturations (16 28)

Rank sum tests correlation and multiple regression *analyses were used for statistical analyses* [1]

RESULTS

No distinction was made for sex and age in the figures quoted since no significant differences were found

Haematological parameters Haemoglobin concentration haematocrit erythrocyte count mean corpuscular volume and mean corpuscular haemoglobin were significantly larger while the MCHC was lower in the diabetic than in the control children The average 2 3 DPG content of the erythrocytes was significantly increased among the diabetic children (Table 2)

2 3 DPG and haemoglobin concentration Fig 1 shows that there was a significant inverse relationship between the 2 3 DPG content and

[1] The statistical analyses were performed in collaboration with lic ing P Thoft Christensen Aalborg

Table 2 *Average haemoglobin erythrocyte count haematocrit mean corpuscular haemoglobin mean corpuscular volume mean corpuscular haemoglobin concentration leucocyte count and 2 3 diphosphoglycerate content (±S D) in diabetic and healthy children*

Groups	Haemoglob (g/100 ml)	Erythrocyt (×10¹²/l)	Haematocrit (volfr)	MCH (fmol)	MCV (fl)	MCHC (mmol/l)
Diabetic children	13 7±0 82	4 8±0 28	0 42±0 03	1 78±0 079	87 9±3 35	20 0±0 71
Healthy children	13 1±0 44	4 6±0 14	0 40±0 02	1 75±0 063	85 3±3 14	20 4±0 68
Significance	$p<0.001$	$p<0.02$	$p<0.001$	$p<0.05$	$p<0.005$	$p<0.05$

Table 3 *Acid–base variables and oxyhaemoglobin saturation in arterialized capillary blood from diabetic and healthy children (average ±S D)*

	Plasma pH	P_{CO_2} (kPa)	Standard bicarbonate (mmol/l)	O_2 saturation (mol/mol)
Diabetics	7 42±0 02	4 89±0 39	3 88±1 0	0 98±0 01
Healthy	7 44±0 03	4 67±0 48	4 32±1 30	0 98±0 01
Significance	$p<0\ 01$	n s	n s	n s

fraction with increased oxygen affinity (decreased oxygen availability) in the red cells of diabetic children (29) As demonstrated by column chromatographic studies of haemoglobin from diabetic subjects such a haemoglobin fraction is actually present (26 31) This haemoglobin fraction A_1 is unique in that it is a glycoprotein with a hexose bound by a Schiff base linkage to the terminal valine in both beta chains of haemoglobin (6 20) The levels of A_1 haemoglobin in children with insulin requiring diabetes ranged from 10% to 22% of total haemoglobin and out of 32 diabetic children a normal concentration of A_1 haemoglobin (7%) was only found in one child (26) Bunn & Briehl (7) have shown that the oxygen affinity of haemoglobin A_1 is little affected by the addition of 2 3 DPG in contrast to ordinary haemoglobin A probably because the presence of the carbohydrate on the N terminal residues of haemoglobin A_1 impairs the binding of 2 3 DPG to the tetrameric haemoglobin Besides the data in the present study of increased concentration of haemoglobin and 2 3 DPG in childhood diabetes the inverse relationship of the mean corpuscular haemoglobin concentration to the 2 3 DPG concentration and to the P_{50} *(in vivo)* would be in keeping with the presence of increased amounts of haemoglobin A_1 in diabetes

In previous studies on 2 3 DPG and ODC in and during recovery from diabetic ketoacidosis we as well as others have found very low concentrations of 2 3 DPG (9 10) In the present paper in non acidotic diabetic children the 2 3 DPG varied from normal to increased concentrations Thus the content of this important ligand determining haemoglobin oxygen affinity fluctuates much more in unstable diabetic than in healthy subjects The elevated level of 2 3 DPG found in the non acidotic diabetic children appears to provide a compensatory mechanism whereby the circulating red cells attempt to decrease the oxygen affinity of haemoglobin The question arises however whether this adjustment is sufficient or whether improved metabolic control with respect to the carbohydrate metabolism might further increase the oxygen release capacity of the erythrocytes

The rate of glycolysis and 2 3 DPG synthesis is increased by increased pH (2 3) In the present study the average pH of the diabetic children was significantly decreased as compared to the healthy children and therefore the pH change does not explain the increased 2 3 DPG level No significant decrease in arterial oxygen saturation was found in this study (Table 3) Thus an arterial hypoxemia cannot be responsible for the increase in 2 3 DPG concentration

During recovery from diabetic ketoacidosis we have presented evidence that the concentration of plasma inorganic phosphate (Pi) is the determining factor for the rate of resynthesis of 2 3 DPG (9 10) In the present study of single determinations the average Pi in the diabetic children was significantly higher than that of the healthy children and the correlation between the 2 3 DPG concentration and the Pi was significant ($p<0\ 05$) It is likely that the effect of inorganic phosphate is on the enzymes phosphofructokinase and glyceraldehyde 3 phosphate dehydrogenase and that these en

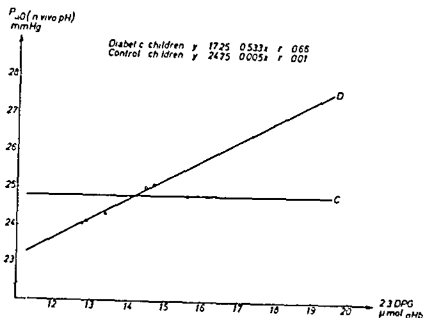


Fig. 2. The correlation between the 2,3-diphosphoglycerate content and the P_{50} (in vivo pH) of the oxyhaemoglobin dissociation curve in diabetic and in healthy children.

between MCHC and P_{50} (7.40) ($r=-0.54$, $p<0.01$).

Acid–base variable and 2,3 DPG. The mean values for P_{CO_2}, pH, standard bicarbonate and oxyhaemoglobin saturation are shown in Table 3. Apart from slightly lower pH among the diabetic children than among the healthy children, there was no significant difference.

DISCUSSION

This study indicates that non-acidotic ambulatory diabetic children have a higher content of red cell 2,3 DPG than normal. This increase was present despite the fact that the average haemoglobin concentration was also significantly increased in the diabetic children.

As indicated in Fig. 1 there was an additional increase in 2,3 DPG content of approximately 4.5 µmol/g haemoglobin at all haemoglobin levels among the diabetic as compared to the healthy children (approximately 30%).

The presence of an elevated haemoglobin concentration and an increased red cell 2,3 DPG content are known adaptive changes to hypoxia. These adjustments have been observed to take place in a number of conditions such as exposure to high altitude (22), anaemia (19, 32), and pulmonary disease (23). Increases in the concentration of 2,3 DPG in the conditions produce corresponding decreases in oxygen affinity of haemoglobin and shifts of the ODC to higher P_{50} values than normal. However, in the present study the increase in 2,3 DPG in the diabetic children did not increase the P_{50} of the ODC above that of the healthy children. The present combination of changes consisting of an elevated haemoglobin concentration, an increased 2,3 DPG in the presence of a normal oxygen affinity and a normal arterial oxygen saturation is characteristic of the presence of abnormal haemoglobins with high oxygen affinity and suggests the existence of such a haemoglobin

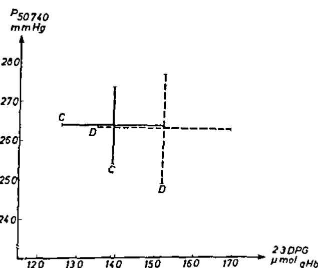


Fig. 3. The correlation between the 2,3-diphosphoglycerate content (mean and standard deviation) and the P_{50} (7.40) (mean and standard deviation) in diabetic (*D*) and in healthy children (*C*).

Table 3 *Acid–base variables and oxyhaemoglobin saturation in arterialized capillary blood from diabetic and healthy children* (average ±S D)

	Plasma pH	P_{CO_2} (kPa)	Standard bicarbonate (mmol/l)	O_2 saturation (mol/mol)
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fraction with increased oxygen affinity (decreased oxygen availability) in the red cells of diabetic children (29) As demonstrated by column chromatographic studies of haemoglobin from diabetic subjects such a haemoglobin fraction is actually present (26 31) This haemoglobin fraction A_{Ic} is unique in that it is a glycoprotein with a hexose bound by a Schiff base linkage to the terminal valine in both beta chains of haemoglobin (6 20) The levels of A_I haemoglobin in children with insulin requiring diabetes ranged from 10% to 22% of total haemoglobin and out of 32 diabetic children a normal concentration of A_I haemoglobin (7%) was only found in one child (26) Bunn & Briehl (7) have shown that the oxygen affinity of haemoglobin A_{Ic} is little affected by the addition of 2 3 DPG in contrast to ordinary haemoglobin A probably because the presence of the carbohydrate on the N terminal residues of haemoglobin A_I impairs the binding of 2 3 DPG to the tetrameric haemoglobin Besides the data in the present study of increased concentration of haemoglobin and 2 3 DPG in childhood diabetes the inverse relationship of the mean corpuscular haemoglobin concentration to the 2 3 DPG concentration and to the P_{50} (*in vivo*) would be in keeping with the presence of increased amounts of haemoglobin A_I in diabetes

In previous studies on 2 3 DPG and ODC in and during recovery from diabetic ketoacidosis we as well as others have found very low concentrations of 2 3 DPG (9 10) In the present paper in non acidotic diabetic children the 2 3 DPG varied from normal to increased concentrations Thus the content of this important ligand determining haemoglobin oxygen affinity fluctuates much more in unstable diabetic than in healthy subjects The elevated level of 2 3 DPG found in the non acidotic diabetic children appears to provide a compensatory mechanism whereby the circulating red cells attempt to decrease the oxygen affinity of haemoglobin The question arises however whether this adjustment is sufficient or whether improved metabolic control with respect to the carbohydrate metabolism might further increase the oxygen release capacity of the erythrocytes

The rate of glycolysis and 2 3 DPG synthesis is increased by increased pH (2 3) In the present study the average pH of the diabetic children was significantly decreased as compared to the healthy children and therefore the pH change does not explain the increased 2 3 DPG level No significant decrease in arterial oxygen saturation was found in this study (Table 3) Thus an arterial hypoxemia cannot be responsible for the increase in 2 3 DPG concentration

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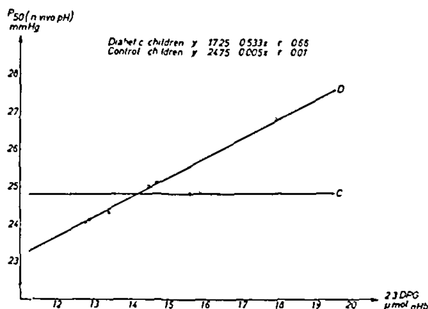


Fig 2 The correlation between the 2,3 diphosphoglycerate content and the P_{50} (in vivo pH) of the oxyhaemoglobin dissociation curve in diabetic and in healthy children

between MCHC and P_{50} (7 40) ($r=-0$ 54 $p<0$ 01)

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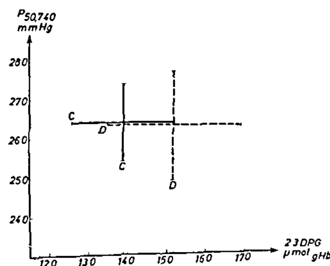


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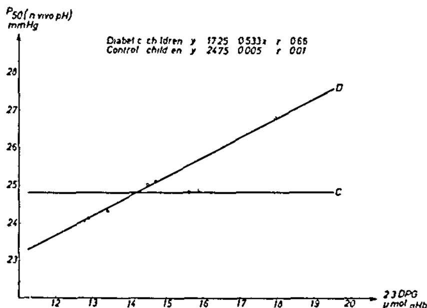


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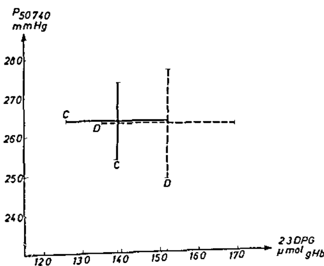


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hemoglobin Effects of carbon dioxide and 2 3 diphosphoglycerate IV Thermodynamical relations between the variables *Scand J Clin Lab Invest* *29* 303 1972

Stamatoyannopoulus G Bellingham A J Lenfant C & Finch C A Abnormal hemoglobins with high and low oxygen affinity *In* A C de Graff (ed) *Annual Review of Medicine* vol *22* Ann Rev Inc Palo Alto 1971 p 221

Travis S E Sugarman H J Ruberg R L Dudrick S J Delivoria Papadopoulos M & Miller L D Alterations of red cell glycolytic intermediates and oxygen transport as a consequence of hypophosphatemia inpatients receiving intravenous hyperalimentation *New Engl J Med* *285* 763 1971

31 Trivelli L A Ranney H M & Lai H T Hemoglobin components in patients with diabetes mellitus *New Engl J Med* *284* 353 1971

32 Valeri C R & Fortier N L Red-cell mass deficits and erythrocyte 2 3 DPG levels *Forsvarsmedicin* *5* 212 1969

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zymes *in vivo* might be in a basically inhibited state (17 27) Also in other clinical situations such as in uremia and during hyperalimentation the concentration of plasma inorganic phosphate has been shown to influence the metabolism of red cells and the affinity of oxygen for haemoglobin (24 30) Thus an optimal oxygen release capacity of the red cells in diabetic children appears to be dependent on a higher than normal level of intraerythrocytic 2 3 DPG and of Pi

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REFERENCES

1 Alberti K G M M Emerson P M Darley J H & Hockaday T D R 2 3 diphosphoglycerate and tissue oxygenation in uncontrolled diabetes mellitus *Lancet II* 391 1972
2 Asakura T Sato Y Minakami S pH dependency of 2 3 diphosphoglycerate content in red blood cells *Clin Chim Acta 14* 840 1966
3 Astrup P Rorth M & Thorshauge C Dependency on acid base status of oxyhemoglobin dissociation and 2 3 diphosphoglycerate level in human erythrocytes II In vivo studies *Scand J Clin Lab Invest 26* 47 1970
4 Bellingham A J Detter J C & Lenfant C The role of hemoglobin affinity of oxygen and red cell 2 3 diphosphoglycerate in the management of diabetic ketoacidosis *Trans Ass Amer Phys 83* 113 1970
5 Benesch R & Benesch R E The effect of organic phosphates from the human erythrocytes on the allosteric properties of hemoglobin *Biochem Biophys Res Commun 26* 162 1967
6 Bookchin R M & Gallop P M Structure of hemoglobin A_1 Nature of the N terminal chain blocking group *Biochem Biophys Res Commun 32* 86 1969
7 Bunn H E & Briehl R W The interaction of 2 3 diphosphoglycerate with various human hemoglobins *J Clin Invest 49* 1088 1970
8 Chanutin A & Curnish R R Effect of organic and inorganic phosphates on the oxygen equilibrium of human erythrocytes *Arch Biochem 121* 96 1967
9 Ditzel J Importance of plasma inorganic phosphate on tissue oxygenation during recovery from diabetic ketoacidosis *Hormone Metabol Res 5* 471 1973
10 — Effect of plasma inorganic phosphate on tissue oxygenation during recovery from diabetic ketoacidosis *Advanc Exp Med Biol 37A* 163 1973
11 — Adaptive changes to tissue hypoxia in ambulatory juvenile diabetics *Diabetes* Suppl 1 2? 317 1973
12 — Increased intraerythrocytic 2,3-diphosphoglycerate oxygen-carrying capacity and regional blood flow in ambulatory juvenile diabetics *Microvasc Research 6* 261 1973
13 Ditzel J Andersen H & Daugaard Peters N Increased haemoglobin A_1 and 2 3-diphosphoglycerate in diabetes and their effects on red cell oxygen releasing capacity *Lancet II* 1034 1973
14 Duvelleroy M A Buckles R C Rosenkaimer S Tung C & Laver M A An oxyhemoglobin dissociation analyzer *J Appl Physiol 28* 227 1970
15 Ericsson Å & de Verdier C H A modified method for the determination of 2 3-diphosphoglycerate in erythrocytes *Scand J Clin Lab Invest 29* 85 197?
16 Garby L Robert M & Zaar B Proton and carbamino linked oxygen affinity of normal human blood *Acta Physiol Scand 84* 482 1972
17 Gerlach E & Duhm J 2 3 DPG metabolism of red cells Regulation and adaptive changes during hypoxia *In* M Rorth & P Astrup (eds) *Oxygen affinity of hemoglobin and red cell acid–base status* Munksgaard Copenhagen 1972 p 552
18 Guest G M & Rapoport S Role of acid soluble phosphorus compounds in red blood cells *Am J Dis Child 58* 1072 1939
19 Hjelm M The content of 2 3-diphosphoglycerate and some other phosphocompounds in human erythrocytes from healthy adults and subjects with different types of anemia *Forsvarsmedicin 5* 219 1969
20 Holmquist W R & Schroeder W A A new N terminal blocking group involving a Schiff base in hemoglobin A_1 *Biochemistry (Wash) 5* ?499 1966
21 Kampen E J van & Zijlstra W G Standardization of hemoglobinmetry *Clin Chim Acta 6* 538 1961
22 Lenfant C Torrance J D English E Finch C A Reynafarje C Ramos J & Faura J Effect of altitude on oxygen binding by hemoglobin and on organic phosphate levels *J Clin Invest 47* 2652 1968
23 Lenfant C Ways P Aucutt C & Cruz J Effect of chronic hypoxic hypoxia on the O_2 Hb dissociation curve and respiratory gas transport in man *Resp Physiol 7* 7 1969
24 Lichtman M A & Miller D R Erythrocyte glycolysis 2 3 diphosphoglycerate and adenosine triphosphate concentration in uremic subjects Relationship to extracellular phosphate concentration *J Lab Clin Med 76* 267 1970
25 Parekh A C & Jung D H Serum inorganic phosphorus determination using p phenylene diamine as a reducing agent *Clin Chim Acta 27* 373 1970
26 Paulsen E P Hemoglobin A_1 in childhood diabetes *Metabolism 22* 269 1973
27 Rapoport S Maretzki D & Schewe C Control of glycolysis in the erythrocyte on the level of 1 3 DPG *In* M Rorth & P Astrup (eds) *Oxygen affinity of hemoglobin and red cell acid–base status* Munksgaard Copenhagen 1972 p 527
28 Siggaard Andersen O Rorth M Norgaard Pedersen B Sparre Andersen O & Johansen E Oxygen linked hydrogen ion binding of human

Table 1 *Main clinical and laboratory findings*

Case	Year of birth	Diagnosis made at age	Age at death	Leuko cyte agglu tinins	Serum protein g/100 ml	Serum globulins g/100 ml	Immuno- globulins	Increase in antibody titre after vaccination (Diphtheria)	Chromosom analysis
M	1956	1 mo	7 mo	–	5 8	1 ?	–	–	–
D	19 8	1 mo	3 mo	–	6 _	1 8	–	–	–
T	1959	3 mo	11 mo	0	7 1	1 7	IgA high	?0×	Normal
W_2	1960	? 5 mo	10 mo	0	6 1	1 0	–	–	–
X	196	1 mo	5 mo	–	5 8	1 0	Normal	–	Normal
X	1964	Day of birth	1 yr	0	6 1	0 8	Normal	–	–
N	1964	1 5 mo	6 5 yr	–	8 7	2 8	IgA, IgG high	?0×	–
P	1965	1 mo	3 5 yr	–	6 8	? 0	Normal	–	–
S	1967	Day of birth	7 mo	0	6 8	2 1	IgA, IgG high	–	Normal
U	1973	? mo	Alive	–	–	–	–	–	–

+=performed –=not performed 0=negative

other developed eosinophilia during treatment with trimethadion

Granulocytopenia in neonates with IGA

In 3 of the new cases blood counts were performed on the first day of life (X_2, S_1 and D_4) In D_4 leukocyte and differential counts were recorded as normal and no further investigations were made before the infant was admitted to hospital with carbunculosis severe pyrexia and agranulocytosis In the other 2 repeated investigations were carried out The leukocyte and granulocyte counts during the first week were as follows

	1st day	3rd day	6th day
X_2	9 200	9 400	9 600
	1 650 (18 %)	1 030 (12 %)	670 (7 %)
S_1	8 400	5 700	8 000
	1 540 (17 %)	450 (8 %)	80 (1 %)

(Norm il findings for the 1st day c 18 000 leukocytes with 60 % granulocytes and for the 7th day 12 000 leukocytes with 45 % granulocytes)

Bone marrow

The marrow smears showed as a rule normal cellularity In the terminal stages the cell count was often low The pattern was characterized by a maturation arrest with block at the *promyelocyte–myelocyte level* The nuclei of these cells were frequently atypical Very few mature cells were observed Erythropoiesis was usually apparently normal although occasionally a slight maturation arrest was seen

Bone marrow cultures

Cell culture on bone marrow from 5 patients (Table I) were performed in fluid medium by a modification of Osgood s technique (26) Erythropoiesis was estimated by the number of erythrocytes produced per time unit Myelopoiesis was estimated by counting mature granulocytes

In all cases the same results were obtained Culture of marrow cells with the childs serum yielded no mature granulocytes but on culture in normal serum granulocytes appeared IC marrow from a normal individual was cultured in serum from a sick child reduced formation of mature granulocytes took place Erythropoiesis was always about 20 % slower than normal when the marrow was cultured in the patient s own serum but on culture in serum from a normal individual it was normal Normal marrow cultured in serum from an IGA patient gave a 10–15 % slower rate of erythrocyte formation

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REVIEW ARTICLE

INFANTILE GENETIC AGRANULOCYTOSIS

A Review with Presentation of Ten New Cases

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ABSTRACT Kostmann R R O (Department of Paediatrics University Hospital Uppsala Sweden) Infantile genetic agranulocytosis Acta Paediatr Scand 64 362 1975 —A review of the literature on the subject since 1956 is made in connection with a presentation of ten new cases from northern Sweden Nine of these are related to the main pedigree published in 1956 Consanguinity between the parents has been established in two of the new families The clinical course was identical to that described in 1956 A few additional details are presented The granulocytopenia is present on the first day of life and the granulocyte count subsequently rapidly decreases during the first week The existence of a diaplacental factor is regarded highly probable It is assumed that the maturation defect in the granulocyte precursors may be due to deficiency of a serum factor The fact that many cases of infantile genetic agranulocytosis occur sporadically is finally explained

KEY WORD Infantile genetic agranulocytosis

Since 1956 when I presented my thesis Infantile Genetic Agranulocytosis (IGA) (15) about 30 patients with similar features have been reported

In northern Sweden 10 new cases have occurred All have been observed and treated at hospitals One patient was born and treated at the University Hospital Uppsala and the others were born and treated at the hospitals in Boden and Gallivare Six of these have also been treated at the Uppsala Hospital and one at the University Hospital Umeå where they were subjected to detailed clinical studies

CLINICAL AND LABORATORY STUDIES

The main clinical and laboratory findings are shown in Table 1

The clinical signs were identical in all cases and are identical to those described in 1956 (15) At a very early age the infants developed bacterial infections Initially cutaneous manifestations with boils and sores were very common and inevitably appeared at some stage of the illness Otitis pneumonia gingivitis and urinary infection were common Septicaemia peritonitis or severe enteritis were common in the terminal stages *Staphylococcus aureus* and *E coli* were the commonest cause of infection During the illness various bacterial strains could be cultured from the sites of infections All patients showed persistent severe granulocytopenia or agranulocytosis and monocytosis In 2 patients there was eosinophilia for a period of 2 weeks One had received three blood transfusions immediately before and during this period The

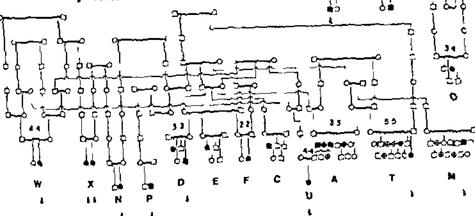


Fig. 1 Pedigrees in infantile genetic agranulocytosis in north Sweden

would be obtained in any patient with marked granulocytopenia and they are probably not pathognomonic of IGA

Case no 4 in the report of McGillivray and collaborators (23) seems uncertain No clinical sign appeared before 2 ½ years of age when she developed pneumonia with pyrexia Initial investigation disclosed 4 600 leukocytes/ mm³ with 34% neutrophils Two months later the patient showed more pronounced granulocytopenia which persisted After the age of 11 years the patient suffered only minor infections and she was 16 years old when the survey was concluded The degree of her granulocytopenia during this 5 year period is not described She never showed skin infection *Their* case no 5 also seems doubtful Admittedly the skin infections appeared early (3 ½ months) but although in chronic benign granulocytopenia the usual age of onset is 1–3 years an even earlier debut has sometimes been described This patient was 11 years when the survey was concluded

The cases published by Gilman and collaborators are remarkable (10) Their case no 1 who from an age of 3 weeks showed grave granulocytopenia with a history and course characteristic of IGA lived unusually long At 14 years the clinical picture became transformed into that of a blastcell leukemia and the child died within 4 months Their case no 3 was the first IGA case in the world in which granulocytopenia at birth could be established Unfortunately no exact details are given about the blood counts on the first day and during the first week of life The history and course of the illness seems compatible with IGA The child was 12 years old and still alive when the survey was finished

The 10 new Swedish cases all showed the clinical picture that was described 1956 and that has since been confirmed by other investigators

It has now been proved that granulocytopenia may exist and probably always exists from the very beginning of extrauterine life Two children showed striking granulocytopenia on the very first day The granulocyte count subsequently rapidly decreased and after a few days fell to a critical

moglobin 0 ml e range	Reticulo cytes % range	Neutro-phils/mm³ range	Eosino phils/mm³ range	Monocytes/mm³ range	Marrow cultures	³H thymidine labelling
-12 0	0- 2 6	0- 900	0-1 400	1 300-6 800	+	-
- 7 0	-	110- 750	0- 400	750-8 000	+	-
- 9 5	0 4- 2 4	40- 90	0- 250	900-5 000	+	-
-12 2	1 6- 5 4	0- 300	0- 300	2 300-7 400	+	-
-13 9	1 2- 5 2	0-1 500	0- 250	650-4 000	+	+
-20 0	0 8- 7 6	0-1 550	0- 650	800-4 500	-	+
-13 2	1 6- 7 8	0- 300	0-1 000	280-2 700	-	+
-15 4	1 3-10 0	0-1 000	0- 900	950-9 600	-	-
-20 4	1 2-11 6	40- 800	0- 150	2 200-4 500	-	-
-13 7	1 3- 3 0	0- 500	0- 400	600-1 300	-	-

³H labelling experiments

In vitro labelling of marrow cells with the DNA precursor Thymidine (³H thymidine) was done in 3 cases (Table 1) Deficient uptake by the neutrophil myelocytes was observed but labeling of the eosinophil myelocytes was normal The results have been published and discussed in detail by Lundmark (22)

Post mortem findings

Necropsy was done in 5 cases Beyond hypocellular bone marrow with mononuclear pattern and normal erythropoiesis the only pathological findings were such as could be explained by infection during a state of agranulocytosis

Genetics

All patients originated from the same region in northern Sweden as the cases published in 1956 Two (D_4 and T_1) belonged to families showing previous incidence of IGA The remaining 8 infants came from seven new families (for pedigrees see Fig 1)

Attempts at therapy

Numerous experiments were carried out—blood transfusion plasma transfusion transfusion of packed white cells non specific fever therapy cortisone testosterone, somatotropic hormone and vitamin B_6 No effect was obtained Antibiotic therapy was the only means of prolonging life All the infants finally succumbed to infection except for the tenth (U_1) who is 11 months old at the time of writing

DISCUSSION

Most cases of IGA published since 1956 in different countries have shown the same clinical features and haematological findings as those from northern Sweden (1 2 3, 4 10 12 14 16 18 19 20 21 23 25 27 30) Some have shown eosinophilia of a degree and persistence that I have never encountered in patients from northern Sweden In southern Sweden two cases of IGA both displaying persistent eosinophilia have recently been found (5 29)

The two cases published by Bjure et al (8) showed neither the clinical course nor the grade of granulocytopenia or marrow pattern that seems to be consistent with IGA However the changes of myelopoiesis in marrow culture were similar to those seen in IGA in northern Sweden Also in a classical case of chronic benign granulocytopenia in an infant in southern Sweden observed by me the same results were obtained Presumably the same results

The IGA cases now presented constitute further evidence that infantile genetic agranulocytosis as observed in a north Swedish population is undoubtedly due to an anomalous autosomal recessive gene Consanguinity is demonstrated in two of the new families (U W) In 6 of them a relationship to the original pedigree of 1956 has been established Especially noteworthy is case U_1 whose father is a brother of the 4 subjects in family A

Some authors find it remarkable that among cases reported outside Sweden so few families have been found to have more than one sick child and in no case has consanguinity been established In fact this is by no means surprising It is extremely difficult to trace rare recessive genes except in isolated geographical areas with excessive inbreeding and an accumulation of homozygotes One such area is the parish of Overkalix Norrbotten Sweden Since the number of children per family in Europe and the United States is usually small most cases of rare recessive illness occur sporadically It is important to remember that when comparing two child families there are theoretically six families with one affected child to only one family where both are affected (and nine with two healthy children of heterozygous parents) Furthermore parents who have already produced an affected child may be disinclined to take the 25% risk once more

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REFERENCES

1 Aarskog D Infantile congenital aneutrocytosis *Arch Dis Child 36* 511 196

Andrews J P McClellan J T & Scott C H Lethal congenital neutropenia with eosinophilia occurring in two siblings *Am J Med* 29 358 1960

3 Barak Y Paran M Leven S & Sachs L In vitro induction of myeloid proliferation and maturation in genetic agranulocytosis *Blood 38* 74 1971

4 Beard M E J Newmark P Smith M E & Franklin A W Infantile genetic agranulocytosis associated with changes in serum vitamin B_{12} binding proteins *Acta Paediatr Scand 61* 5?6 1972

5 Berglund G Personal communication

6 Bierman H R Characteristics of leukopoietin G in animals and in man *Ann N Y Acad Sci 113* 15 1964

7 Bierman H R Marshall G J Mackawa T & Kelly K H Granulocytic activity of human plasma *Acta Haematol* (Basel) 27 ?17 196?

8 Bjure J Nilsson L R & Plum C M Familial neutropenia possibly caused by a deficiency of plasma factor *Acta Paediatr Scand 51* 497 196_

9 Chan S H Metcalf D & Stanley E R Stimulation and inhibition by normal human serum of colony formation in vitro by bone marrow cells *Br J Haematol* 20 3_9 1971

10 Gilman P A Jackson D P & Guild H G Congenital agranulocytosis Prolonged survival and terminal acute leukemia *Blood 36* 576 1970

11 Hall C A Transport of vitamin B_{12} in man *Br J Haematol 16* 4 9 1960

12 Hedenberg F Infantile agranulocytosis of probably congenital origin *Acta Paediatr Scand 48* 77 1959

13 Killander A The serum vitamin B_{12} levels at various ages *Acta Paediatr Scand 46* 585 1957

14 Kriker W T & Panos T C Idiopatic infantile agranulocytosis with hypergammaglobulinemia *Amer J Dis Child 94* 549 1957

15 Kostmann R R O Infantile genetic agranulocytosis *Acta Paediatr Scand* Suppl 105 1956

16 Krill C E & Mauer A M Congenital agranulocytosis *J Pediatr* 68 361 1968

17 Kumento A Studies on the serum binding of vitamin B_{12} in the newborn human infant *Acta Paediatr Scand* Suppl 194 1969

18 Kurz R Glatzl J & Schmalzl F Congenital agranulocytosis Case report and pathogenetic study *Paediatr Paedol 6* 75 1971

19 Larsen H W Extreme neutropenia occurring in an infant *Acta Paediatr Scand 43* 636 1957

0 Lang J E & Cutting H O Infantile genetic agranulocytosis *Pediatrics 35* 596 1965

1 Luhby A L Speer F D Lee R & Shapiro A O Congenital genetic agranulocytosis *Am J Dis Child 94* 55 1957

Lundmark K M Bone marrow cell proliferation in health and in haematological disease during childhood *Acta Paediatr Scand* Suppl 16? 1966

_3 MacGillivray J B Dacie J V Henry J R K Sacker L S & Tizard J P M Congenital neutropenia A report of five cases *Acta Paediatr Scand 53* 188 1964

4 Metcalf D Clinical applications of the agar culture technique for haematopoietic cells *Rev Eur Etud Clin Biol 16* 855 1971

5 Miller D R Freed B A & Lapey J D Congenital neutropenia *Am J Dis Child 115* 33? 1968

?6 Plum C M Methods for continuous tissue culture as applied to bone marrow *Acta Physiol Scand 11* ?60 1946

?7 Rodin A E Haggard M E Nichols M M &

level This observation seems to indicate the existence of a diaplacental factor It seems likely that such a factor at any rate to some degree would stimulate myelopoiesis in the foetus

Nine of the new cases died at an early age The tenth is alive 11 months old In the light of present knowledge of IGA it is very doubtful whether case K and L_1 both of whom were described and discussed in 1956 (15) actually suffered from IGA They showed only transient granulocytopenia and their bone marrow pattern never showed the grave arrest of maturation that today must be considered as characteristic of IGA Both are still alive and well They are therefore not included in the new up-to-date pedigrees embracing all known cases of IGA from Northern Sweden (Fig 1)

The ^{3}H labelling experiments *in vitro* using bone marrow cells showed that the defect was localized to DNA synthesis in neutrophil myelocytes Similar results have been obtained by other investigators (18 28 30)

The results obtained in cell cultures of bone marrow from 5 of the new cases one (R_4) from 1956 (15) and Hedenberg s case from 1959 (12) suggest that the blood serum probably either lacked some factor that normally stimulates myelopoiesis or it contained some substance with an inhibitory effect Also a moderate but convincing disorder of erythropoiesis affecting the rate of production of mature erythrocytes was recorded This finding is from clinical point of view somewhat confusing The anemia shown by IGA patients is normochromic and compatible with the assumption that it is induced by the infection The mechanism of the anaemia in children caused by infections is obscure It may be that the slowing down of erythrocyte production by 20% seen in IGA patients (*in vitro*) is a reflection of some toxic factor in the serum induced by the infection It is hard to believe that the deficit in erythrocyte production could be an effect of the same factor that influences myelopoiesis

Since 1966 a new technique for culturing haematopoietic cells has been introduced (24) It is capable of providing valuable information on myelopoiesis Barak and collaborators have studied a classical case of IGA with this new technique (3) Their findings indicate that the block in granulocyte maturation is not related to an inhibitory factor in the blood They assume that in IGA patients there is an inherent deficiency of a substance that induces maturation This substance is in all probability the colony stimulating factor (CSF) described by Metcalf and Stanley (9 24) and most likely the factor described by Bierman (6 7) as Leukopoietin G Bierman succeeded in extracting this factor from bovine kidney and spleen He also demonstrated the factor in blood plasma but in lower concentration than in his tissue extracts The stimulatory effect on myelopoiesis elicited *in vitro* using normal plasma must be interpreted as an effect of that plasma factor However attempts at treating IGA patients with plasma transfusions were always unsuccessful

Some workers have demonstrated that mature neutrophil granulocytes in their granules form and discharge into the plasma those globulins (Transcobalamines TC I TC II and fetal TC) that bind and transport vitamin B_{12} If radioactive vitamin B_{12} is added to neonatal serum *in vitro* 10% of the radioactivity will be bound to TC II and the rest to TC I and/or fetal TC (11 17) Beard and collaborators report radioisotopic vitamin B_{12} studies in a classical case of IGA (4) They showed a probable total absence of fetal TC and marked reduction of serum TC I The vitamin B_{12} concentration in serum was 208 pg/ml which is far below normal (13) Estimation of vitamin B_{12} was done in one of the new cases of IGA (X_1) The B_{12} concentration was 275 pg/ml which too is low

The new findings on vitamin B_{12} and IGA are intriguing but it is hard to believe that a deficit in cobalamines resulting from granulocytopenia and a consequent depression of vitamin B_{12} concentration could be the explanation of the reduced rate of erythropoiesis found *in vitro* in cases of IGA

The IGA cases now presented constitute further evidence that infantile genetic agranulocytosis as observed in a north Swedish population is undoubtedly due to an anomalous autosomal recessive gene Consanguinity is demonstrated in two of the new families (U W) In 6 of them a relationship to the original pedigree of 1956 has been established Especially noteworthy is case U, whose father is a brother of the 4 subjects in family A

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REFERENCES

1 Aarskog D Infant le congenital aneutrocytosis *Arch D s Cl ild* 36 511 196
Andrews J P McClellan J T & Scott C H Lethal congen tal neutropenia with eosinophilia occurring in two siblings *Am J Med* 29 358 1960
3 Barak Y Paran M Leven S & Sachs L In vitro induction of myeloid proliferation and maturation in genetic agranulocyto is *Bl od* 38 74 1971
4 Beard M E J Newmark P Smith M E & Franklin A W Infantile genetic agranulocytosis associated with changes in serum vitamin B_{12} binding proteins *Acta Paediatr Scand* 61 576 1972
5 Berglund G Personal communication
6 Bierman H R Characteristics of leukopoietin G in animals and in man *Ann NY Acad Sci* 113 15? 1964
7 Bierman H R Marshall G J Mackawa T & Kelly K H Granulocytic activity of human plasma *Act i Haematol* (Basel) 27 217 1962
8 Bjure J Nilsson L R & Plum C M Familial neutropenia possibly caused by a deficiency of plasma factor *Acta Paediatr Scand* 51 497 1962
9 Chan S H Metcalf D & Stanley E R Stimulation and inhibition by normal human serum of colonyformation in vitro by bone marrow cells *Br J Haematol* 20 329 1971
10 Gilman P A Jackson D P & Guild H G Congenital agranulocytosis Prolonged survival and terminal acute leukemia *Blood* 36 576 1970
11 Hall C A Transport of vitamin B , in man *Br J Haematol* 16 4 9 1960
1_ Hedenberg F Infantile agranulocytosis of probably congenital origin *Acta P ediatr Scand* 48 77 1959
13 Killander A The serum vitamin B levels at various ages *Acta Paedi itr Scand* 46 585 1957
14 Kniker W T & Panos T C Idiopatic infantile agranulocytosis with hypergammaglobulinemia *Amer J Dis Child* 94 549 1957
15 Kostmann R R O Infantile genetic agranulocytosis *Acta Paediatr Sc ind* Suppl 105 1956
16 Kn ll C E & Mauer A M Congenital agranulocytosis *J Pediatr* 68 361 1968
17 Kumento A Studies on the serumbinding of vitamin B , in the newborn human infant *Acta Paediatr Scand Suppl* 194 1969
18 Kurz R Glatzl J & Schmalzl F Congenital agranulocytosis Case report and pathogenetic study *Paedi itr P iedol* 6 75 1971
19 Larsen H W Extreme neutropeni i occurring in an infant *Acta Paediatr Scand* 43 636 1957
_0 Lang J E & Cutting H O Infantile genetic agranulocytosis *Pediatrics* 35 596 1965
?1 Luhby A L Speer F D Lee R & Shapiro A O Congenital genetic agranulocytosis *Am J Dis Child* 94 557 1957
? Lundmark K M Bone marrow cell proliferation in health and in haematological disease during childhood *Acta P iediatr Scand* Suppl 16 1966
?3 MacGillivray J B Dacie J V Henry J R K Sacker L S & Tizard J P M Congenital neutropenia A report of five cases *Act i Paedi itr Scand* 53 188 1964
?4 Metcalf D Clinical applications of the agar culture te hnique for haematopoietic cells *Rev Eur Et id Clin Biol* 16 855 1971
25 Miller D R Freed B A & Lapey J D Congenital neutropenia *Am J Dis Cl i d* 115 337 1968
?6 Plum C M Methods for continuous tissue culture as applied to bone marrow *Acta Physiol Scan l* 11 60 1946
?7 Rodin A E Haggard M E Nichols M M &

Gustavson P P Infantile genetic agranulocytosis *Am J Dis Child 126* 818 1973
28 Ruvidic R Nahean Y Varet B & Bernard J Study of granulocytopoiesis by autoradiography of bone marrow in vitro *Nouv Rev Fr Hematol 10* 505 1970
29 Thilén A Personal communication
30 Wried K Kauder E & Mauer A M Defective myelopoiesis in congenital neutropenia *N Engl J Med* 28? 1072 1970

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CASE REPORT

MANIFEST INTESTINAL INVOLVEMENT DURING BONE MARROW REMISSION IN A CASE OF ACUTE LYMPHOBLASTIC LEUKAEMIA

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ABSTRACT Aronson A S Garwicz S Landberg T Nelson Ö and Brun A (Departments of Paediatrics Radiotherapy Paediatric Surgery Pathology University Hospital Lund Sweden) Manifest intestinal involvement during bone-marrow remission in a case of acute lymphoblastic leukaemia Acta Paediatr Scand 64 369 1975.—Massive leukaemic involvement of the intestine appeared in a 9 year-old girl with acute lymphoblastic leukaemia The unusual feature in this case was that the gut involvement occurred during complete haematological remission Surgical and subsequent radiological treatment completely eradicated the engagement and at autopsy 9 months later there were no signs of the intestinal involvement

KEY WORDS Intestinal involvement leukaemia

In the active phase of acute leukaemia wide spread infiltration of extramedullary sites is well known to occur In therapeutic failures massive involvement is seen in several organs including the gastrointestinal tract (5) Extramedullary leukaemic foci are also seen in haematological remission Apart from the well documented leukaemic engagement of the central nervous system infiltration of other organs has been described in autopsy material (3 4 7) as well as in living patients (2 6) Thus in 12 of 31 patients (18 children) with blood and bone marrow remission according to routine tests Mathe et al (2) found the persistence of leukaemic cells in one or several of the following sites blood bone marrow kidney liver and testicles In autopsy material from patients with bone marrow remission the frequency of gastro intestinal infiltration was found to be 0–30% (3 4 7) These infiltrations were asymptomatic during the patient s life

In the present report we give an account of a patient with acute lymphoblastic leukaemia in haematological remission who experienced dramatic symptoms of an extensive leukaemic gut infiltration which responded favourably to treatment

CASE REPORT

D K 63 04 30 This girl fell ill in December 1968 *at the age* of 5½ years and acute lymphoblastic leukaemia was diagnosed At this time her Hb was 5 0 g/100 ml WBC 2 700/mm platelets 160 000/mm There was no palpable enlargement of the *liver spleen* or peripheral lymph nodes Bone marrow remission was induced by Vincristine and Prednisolone Mercaptopurine was given for maintenance treatment Haematological relapse occurred 3 years later in February 1972 with 50% lymphoblasts in the bone marrow Remission was again *obtained* with Vincristine and Prednisolone For maintenance Methotrexate was given twice weekly Haematological remission was verified





Fig 1 Small intestine with leukaemic infiltration restricted to the mucosa Ulceration shown in top centre of the picture Hematoxylin and eosin ×15

Fig 2 Detail of infiltration shown in Fig 1 occasional preserved mucosal gland surrounded by lymphoblastic and reticulum cells Hematoxylin and eosin ×400

by repeated bone marrow aspirations from the crista iliaca March 14 April 4 June 28 August 4 September 6 October 11 November 16 and from the sternum September 15 1972 During periods of radiotherapy cytostatic treatment was temporarily abandoned

Central nervous system involvement

The first symptoms of CNS engagement appeared in June 1969 The patient was treated with Methotrexate intrathecally until remission and then maintained on the same drug with every sixth week In spite of this treatment the girl experienced repeated CNS relapses which responded to intensified intrathecal treatment and later on to craniospinal radiation therapy In December 197? symptoms and CSF findings indicated CNS leukaemic relapse Radiotherapy was given in February 1973 to the cerebrum and the CNS findings normalized In the last 2 months of her life the patient developed signs of progressive cerebral deterioration

Gut involvement

In July 197? the patient suffered from periods of vomiting poor appetite abdominal discomfort and frank pain Her body weight decreased from 3 5 kg to 0 5 kg On August 1 her condition deteriorated Following haematemesis symptoms of peritonitis developed Laparotomy was performed on the same day Abundant green somewhat foul smelling fluid was found in the abdominal cavity Several plaque like hard thickenings were noticed in the wall of the small intestine mainly in the jejunum No such indurations could be felt in the colon The stomach and duodenum were normal on palpation and no perforations were visible The liver and the spleen had smooth surfaces and were not significantly enlarged A large part of the ileum was situated deep in the pelvis forming a conglomerate adherent to the pelvic wall and the bladder In the mesentery of the small intestine several large lymph nodes were found Three patchy foci of necrosis about 1 cm in diameter were located in the centre of the plaque like thickenings and four jejunal perforations some of them covered by the omentum were found A wedge resection of the jejunum was performed and the remaining smaller foci of necrosis were oversewn

Histological investigation of necrotic as well as macroscopically intact tissue revealed a diffuse infiltration by malignant lymphoblast like cells (Figs 1 and 2) The same picture was found in a lymph node taken from the mesentery of the small intestine Bone marrow aspiration performed ? days later showed normal cellularity and no identifiable leukaemic cells A biopsy *ad modum* Daniels ? weeks after operation revealed normal histology of the lymph node

Postoperatively the patient received one injection of Vincristine and radiation therapy to the abdomen was given One ventral [60]Cobalt field at 70 cm source skin distance was used The field (size 14×19 cm) included the abdomen down to the floor of the pelvis and up to the xiphoid Later the field was diminished to in lude only the caudal part of the initial target The total absorbed dose at a depth of 5 cm from the surface was for the cranial part of the field 900 rad given in 14 fractions in ?1 days (nominal standard dose NSD_{60C} =330 ret) and for the caudal part of the field 1 300 rad given in 0 fractions in 34 days (NSD_{60C} =430 ret) Full parenteral nutrition for the first ? weeks was followed by step-wise introduction of oral nutrition The postoperative course was uneventful apart from disturbing nau ea and vomiting after each radiation dose The intestines functioned well and the appetite increased successively The patient gained weight 3 kg in one month Radiological examination of the gastrointestinal tract 6 weeks after operation revealed no morphological or functional abnormalities Rectoscopy was performed on October 17 and showed normal findings Microscopic investigation of the rectal biopsy showed no leukaemic infiltration In the following course of the disease the patient showed no signs or symptoms attributable to leukaemic involvement of the intestines

On March 19 1973 a haematological relapse was diagnosed by bone marrow asp ration After one month of inductic n therapy the patient expired because of an intercurrent infection

Po t m rtem examinati n showed no leukaem c infiltration in the bone marrow lymph glands liver or spleen on microscopic examination At the site of the small intestine resection there was a narrow scar and the intestinal mucosa was of natural appearance with normal Peyer s patches Microscopically there was no leukaemic infiltration of the gut

DISCUSSION

Among the organs actively involved despite bone marrow remission intestinal infiltration is very seldom Most reports concern autopsy material and gastrointestinal engagement remains undiscovered during the patient s life being apparently asymptomatic (3 4) Only Everett et al (1) have reported on a child with acute leukaemia succumbing to the complications arising from extensive infiltration of the gastrointestinal tract Bone marrow examination 9 days prior to death showed a complete remission At autopsy leukaemic infiltrations were present in the lungs liver and spleen but unfortunately the result of the post mortem bone marrow examination was not reported

Our patient presented a life threatening condition originating in peritonitis due to perforations of leukaemic infiltrations of the intestine during complete bone marrow as well as central nervous system remission The marrow remission was confirmed by repeated aspirations and lasted 7½ months from the time of diagnosis of gut involvement The reported case illustrates the potential curability of extensive leukaemic infiltration of the intestine

The pathogenesis of isolated (extensive) leukaemic infiltration of the gut—as well as of other parenchymatous organs—during marrow remission in puzzling In contrast to central nervous system leukaemia which is apparently explainable by the inaccessibility for the systemic treatment the isolated intestinal involvement must engage other mechanisms

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REFERENCES

1 Everett C R Haggard M E & Levin W C Extensive leukaemic infiltration of the gastrointestinal tract during apparent remission in acute leukaemia *Blood 22* 92 1963
2 Mathé G Schwarzenberg L Mery A M Cattan A Schneider M Amiel J L Schlumberger J R Poisson J & Wajcner G Extensive histological and cytological survey of patients with acute leukaemia in complete remission *Br Med J 1* 640 1966
3 Nies B A Bodey G P Thomas L B Brecher G & Freireich E J The persistence of extramedullary leukaemic infiltrates during bone marrow remission of acute leukaemia *Blood 26* 133 1965
4 Pluss H J & Johnson W W Residuelle Organinfiltrate bei behandelten Leukämien im Kindesalter *Schweiz Med Wochenschr 102* 1200 1972
5 Prolla J C & Kirsner J B The gastrointestinal lesions and complications of the leukaemias *Ann Intern Med 61* 1084 1964
6 Sharp H L Nesbit M E D Angio G J & Krivit W Addition of local radiation after bone marrow remission in acute leukaemia in children *Cancer 20* 1403 1967
7 Simone J V Holland E & Johnson W Fatalities during remission of childhood leukaemia *Blood 39* 759 1972

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CASE REPORT

RENAL VEIN THROMBOSIS IN NEONATES

Report of Three Cases Treated with Nephrectomy

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ABSTRACT Halvorsen J F and Moe P J (Department of General Surgery and Department of Paediatrics Haukeland sykehus University of Bergen Bergen Norway) Renal vein thrombosis in neonates Report of 3 cases treated with nephrectomy Acta Paediatr Scand 64 373 1975.—Three neonates with unilateral renal vein thrombosis successfully treated with nephrectomy are reported One of them also received Heparin therapy before and after surgery because of evidence of consumptive coagulopathy All were well at follow up

KEY WORDS Renal vein thrombosis neonates consumptive coagulopathy nephrectomy

Renal vein thrombosis may occur at any age but about 80% of cases are seen in the paediatric age group the peak incidence (60%) being in the neonatal period (7) In some cases the thrombosis apparently starts *in utero* (15) In 1932 Gruneberg (5) reported the first case successfully treated with nephrectomy During the following years nephrectomy was the treatment re commended by most authors (1 3 6 7 10 12) In 1949 Fallon (4) reported a case with survival following bilateral renal vein thrombosis treated conservatively Later many reports have appeared advocating conservative therapy (2 9 11 13 14) The present paper reports 3 cases of unilateral renal vein thrombosis in neonates success fully treated with nephrectomy during the period 1968–72

Case Histories

The case histories are presented in Table 1

DISCUSSION

Thrombosis of the renal vein may occur uni or bilaterally and as a rule results in haemorrhagic infarction of the kidney Currently primary and secondary types can be distinguished The primary type occurs soon after birth is rapid and involves the main renal vein and its larger tributaries (12) Our cases 1 and 2 probably belong to this type The secondary type is in most cases associated with a well defined patho logical process such as pyelonephritis It often starts in the intrarenal radicles progresses slowly and is often bilateral (12) Our case 3 appears to be representa tive of this type

The etiology of renal vein thrombosis is still unsettled and probably rather complex (10 15) The observation by some authors (6 9) that renal vein thrombosis may be associated with thrombi elsewhere in the

Table I *Case histories*

Case	Sex	Age at admission	Symptoms and signs	Laboratory findings	Intravenous pyelogram	Therapy
1	F	4 days	Episode of cyanosis and superficial respiration Palpable flank tumour	Leucocytosis albuminuria hematuria	No excretion on the right side	Preoperative diagnosis Wilms tumour Right sided nephrectomy (Age 6 days)
2	F	8 days	Acute illness with anorexia Blood and mucus in napkins Palpable flank tumour	Leucocytosis albuminuria hematuria Ethanol test +++ Fibrinogen low Thrombocytopenia	No excretion on the right side	Preoperative diagnosis Renal vein thrombosis Heparin 6 days Right sided nephrectomy (Age 21 days)
3	M	56 days	Anorexia hypotonia diarrhoea Palpable flank tumour	Leucemoid blood picture Thrombocytopenia Hematuria albuminuria pyuria Blood culture *E coli*	Not done because of uremia	Preoperative diagnosis Wilms tumour with secondary infection Sepsis therapy Left sided nephro adrenalectomy (Age 57 days)

vascular system suggests a general tendency to thrombosis in these patients The diagnosis may be suspected on clinical symptoms namely enlargement of one or both kidneys hematuria albuminuria and oliguria/anuria Intravenous pyelography shows enlargement of the renal shadow and no excretion of contrast on the affected side (1 6 7 9 10) Retrograde pyelography may reveal an irregular outline and incomplete filling of the renal pelvis and extravasation of contrast into the renal parenchyma (3) Aortography and selective renal angiography show spreading of the intrarenal arteries slowing of blood flow and decreased nephrographic flush (8) Thrombi may be demonstrated in the inferior vena cava or the renal vein by cavography or retrograde phlebography respectively (8) In two of our cases intravenous pyelography showed no excretion of contrast on the affected side while in one case the examination was not done because of uremia Intravascular coagulation leads to consumption of clotting factors (11) Studies of the clotting factors were done in case 2 and showed consumption coagulopathy

Other conditions to be considered in the differential diagnosis are Wilms tumour hydronephrosis polycystic kidney and perinephritic abscess or hematoma (6 9 10) In two of our cases the preoperative diagnosis was Wilms tumour

The treatment of renal vein thrombosis is still under debate Campbell & Matthews (3) stated that without prompt surgical intervention the mortality rate would be 95% whereas a survival rate of 75% might be expected following emergency nephrectomy During recent years many authors have challenged this statement (2 4 9 11 13 14) The advocates of conservative treatment state that in more than half of the nephrectomy cases the patients were in no imminent danger and were in fact improving on conservative management at the time of the operation and that some of the removed kidneys might have recovered

Pathology	Course
Hemorrhagic necrosis of the kidney due to thrombosis of the main renal vein No inflammation or intra renal thrombosis	Uneventful Normal at the age of 1 year
Hemorrhagic necrosis of the kidney Thrombosis of main renal and intra renal veins more advanced in intra renal veins	Uneventful Normal at the age of 1 year
Hemorrhagic necrosis of the kidney and adrenal gland Acute pyelonephritis with microabscesses Thrombosis of main renal and intrarenal veins without signs of organisation	Uneventful Normal at the age of 1 year

their function if left in place (14) However at least some of the nephrectomy cases were held to be in a desperate condition prior to surgery and showed dramatic improvement after removal of the thrombosed kidney (6 7 12) Though none of our patients was in a desperate condition all were obviously ill and showed marked improvement following nephrectomy In two cases the nephrectomy was done urgently 1 and 2 days after admission respectively The third patient was operated electively 13 days after admission following a trial of anticoagulant therapy

Judging from the extent of damage in the pathological specimens it seems unlikely that the kidneys in case 2 and 3 could have recovered any significant function Case 1 might have benefited from conservative therapy

REFERENCES

1 Beck D & Marshall V F Renal venous thrombosis in children *Aust N Z J Surg 38* 35 1968

2 Belman A B Susmano D F Burden J J & Kaplan G W Nonoperative treatment of unilateral renal vein thrombosis in the newborn *JAMA 211* 1165 1970

3 Campbell M F & Matthews W F Renal thrombosis in infancy Report of two cases in male infants urologically examined and cured by nephrectomy at thirteen and thirty three days of age *J Pediatr 20* 604 1942

4 Fallon M L Renal venous thrombosis in the new born *Arch Dis Childh 24* 125 1949

5 Gruneberg B Nierenvenenthrombose linkerseits *Munch Med Wschr 79* 1054 1932

6 Karafin L & Stearns T M Renal vein thrombosis in children *J Urol* 92 91 1964

7 Kaufmann H J Renal vein thrombosis *Am J Dis Child 95* 377 1958

8 Kees C J & Harrell R S Radiographic manifestations of renal vein thrombosis *J Urol 108* 830 1972

9 McFarland J B Renal venous thrombosis in children *Q J Med 34* 269 1965

10 Milburn C L Hemorrhagic infarction of the kidneys in infants Report of a unilateral case in an 8-day old male infant with survival following successful nephrectomy *J Pediatr 41* 133 1952

11 Renfield M L & Kraybill E N Consumptive coagulopathy with renal vein thrombosis *J Pediatr 82* 1054 1973

12 Sandblom P Renal thrombosis with infarction in the newborn Two different forms *Acta Paediat Scand 35* 160 1948

13 Seeler R A Kapadia P & Moncado R Nonsurgical management of thrombosis of bilateral renal veins and inferior vena cava in the newborn infant *Clin Pediatr* 9 543 1970

14 Stark H Renal vein thrombosis in infancy Recovery without nephrectomy *Am J Dis Child 108* 430 1964

15 Takeuchi A & Benirschke K Renal venous thrombosis of the newborn and its relation to maternal diabetes Report of 16 cases *Bi Neonat 3* 237 1961

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NEW BOOKS RECEIVED

M Breunung & M Breunung *Die Harnwegeinfektion Grundlagen für Profylaxe Diagnostik Therapie und Dispensairebetreuung* 353 pp illus VEB Georg Thieme Leipzig 1974 No price given

L D Longo & H Bartels (eds) *Respiratory gas exchange and blood flow in the placenta* 570 pp illus Proceedings of a Symposium in Conjunction with the XXV International Congress of Physiological Sciences Hannover Germany August 4–6 1971 DHEW Publication No (NIH) 73 361 $6 05

G V Skurkovich *Lesions in the upper respiratory tract in blood dyscrasias* 138 pp illus John Wiley & Sons Ltd Chichester 1974 £7 50

J Brostoff (ed) *Clinical immunology-allergy in paediatric medicine* 176 pp illus Scientific Proceedings of the 1st Unigate Paediatric Workshop Royal College of Physicians London June 1973 Blackwell Scientific Publications Oxford London Edinburgh and Melbourne 1973 Price not given

M Iivanainen *A study on the origins of mental retardation* Clinics in Developmental Medicine No 51 172 pp illus Spastics International Medical Publications William Heinemann Medical Books Ltd London 1974 £4 40

J Cravioto L Hambraeus & B Vahlquist (eds) *Early malnutrition and mental development* 87 pp illus Symposia of the Swedish Nutrition Foundation XII (Including papers from an ensuing WHO Workshop) Saltsjöbaden Sweden August 20–22 1973 Almqvist & Wiksell Stockholm 1974 Sw kr 75 —

V C Kelley (ed) *Metabolic endocrine and genetic disorders of children* (Three Volumes) 1841 pp illus Harper & Row Publishers Inc Hagerstown 1974 $125 —

N Kretchmer & E G Hasselmeyer (eds) *Horizons in perinatal research Implications for clinical care* 193 pp illus Symposium Stanford California *In* the Series Clinical Pediatrics Maternal and Child Health John Wiley & Sons New York London Sydney Toronto Chichester 1974 £8 25

S Raiti (ed) *Advances in human growth hormone research* 961 pp illus Symposium Baltimore Maryland October 9–12 1973 DHEW Publication No (NIH) 74-612 Price not given

M Winick (ed) *Nutrition and fetal development* 188 pp illus John Wiley & Sons New York London Sydney and Toronto 1974 £7 70

O A Zorab (ed) *Scoliosis and muscle* 209 pp illus S I M P Research Monograph No 4 Proceedings of a Fourth Symposium The Cardiothoracic Institute Brompton Hospital London November 14–15 1973 Spastics International Medical Publications London 1974 William Heinemann Medical Books Ltd £4 50

Acta Pædiatr Scand 64 377–384 1975

UTILIZATION OI I AT EMULSION DURING TOTAL PARENTERAL NUTRITION IN CHII DREN

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ABSTRACT Forget P Fernandes J and Haverkamp Begemann P (Department of Paediatrics Sophia Children s Hospital and Neonatal Unit Erasmus University Rotterdam the Netherlands) Utilization of fat emulsion during total parenteral nutrition in children Acta Paediatr Scand 64 377 1975.—Tolerance for Intralipid fat emulsion during total parenteral nutrition (PN) was studied in 6 children The Intralipid dose was monitored by the daily determination of plasma Intralipid levels Fat removal was investigated at the start of and during the PN period by the intravenous fat tolerance test (IVFTT) and by determining the plasma postheparin lipoprotein lipase (LPL) activity When the plasma Intralipid levels exceeded a value of 100 mg/100 ml hyper pre β lipoproteinaemia hypertriglyceridaemia hypercholesterolaemia and hyperphospholipidaemia appeared During PN most patients showed marked increases of postheparin LPL. Return to normal values occurred after discontinuation of PN Maximal LPL activities were found to correlate significantly with total daily caloric intake ($r=0.95$ $0.05<p<0.01$) The Intralipid elimination constant hardly changed during PN with the exception of patient 6 who showed a marked increase (from 7 to 22%) Conclusions of this study are as follows First a high caloric intake during PN leads to a marked increase of postheparin LPL activity Second by monitoring plasma Intralipid levels at 100 mg/100 ml approximately it is possible to adjust the Intralipid dose in order to prevent hyperlipaemia and to take maximal benefit from rising fat tolerance Thirdly the IVFTT appeared to be of little value to estimate the child s fat elimination capacity

KEY WORDS Parenteral nutrition hyperlipaemia postheparin lipoprotein lipase intravenous fat tolerance test

During the last few years total parenteral nutrition has been used on a wide scale in paediatric patients

The more generalised use of fat emulsions in intravenous feeding programs (1 12) has made it possible to infuse solutions through peripheral scalp veins for prolonged periods The use of small peripheral veins instead of a central line via a catheter located into a major calibre vein has well known advantages Phlebitis can early be detected and the risk for catheter related complications can be avoided Apart from the practical advantage of including fat emulsions in parenteral nutrition there are also theoretical reasons to justify its use

1 The presence of fat in the intravenous fluid prevents the development of an essential fatty acid deficiency which otherwise develops rapidly during PN (16)

2 It facilitates hypercaloric feeding because of the high calorie/volume ratio of fat emulsions

3 It precludes infusion of unphysiologically high amounts of carbohydrates and amino acids otherwise needed to maintain a calorically adequate regimen

Both the advantages mentioned and the very low incidence of clinical side effects associated with the use of Intralipid (Vitrum) fat emulsion in adult patients (11) make its use in children

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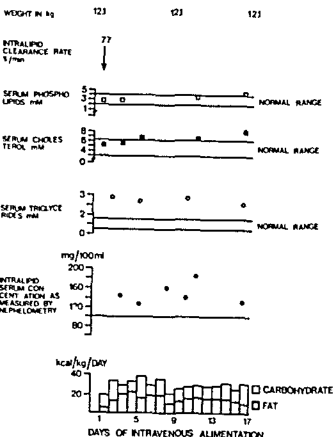


Fig. 1. Total intravenous alimentation of a girl of 2 years with encephalitis. Aminoacids were not administered because it was necessary to restrict the volume intake.

attractive. However, higher caloric needs expressed on a weight basis in children could lead to the use of a higher fat dose/kg body weight than in adults. Are these high doses devoid of clinical side effects? Is it possible to determine the fat tolerance of an individual child in order to adjust the quantity to be infused to the quantity he can tolerate. Little is known about the utilization and tolerance of fat emulsions in paediatric patients. The same Intralipid dose per kg body weight per day has been used by some authors with acute side effects (20) and by others without toxic effects (1). As we have stated earlier (7) toxicity seems to be associated with high Intralipid blood levels. High blood levels are found when the capacity of the enzyme lipoprotein lipase for fat elimination is exceeded. This happens when the Intralipid blood level exceeds a concentration of 100 to 150 mg/100 ml (10).

At this point when the enzyme (LPL) is saturated with its substrate (Intralipid), excessive fat doses are no longer eliminated by the physiological enzymatic removal mechanism but by aspecific uptake in RES and liver (20).

In our preliminary study (7) in one patient we found that LPL is an inducible enzyme. The present paper reports the study of six patients, the first one included. Elimination capacity for fat was frequently checked during the PN period. Intralipid blood levels were checked daily and the Intralipid dose adjusted in order to obtain an Intralipid blood level of 100 to 150 mg/100 ml (LPL saturation level). Frequent controls of serum triglycerides, cholesterol concentrations and of liver function tests were made. Free fatty acids and ketone bodies serum levels were followed in 2 patients.

MATERIAL AND METHODS

Patients and infusion method

Six patients were studied. We administered Intralipid 0% (Vitrum), Vamin (Vitrum) and glucose from separate bottles simultaneously at a constant rate around the clock

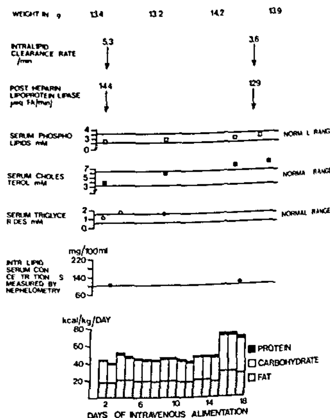


Fig. 2. Total intravenous alimentation of a girl of 4 years with cerebral

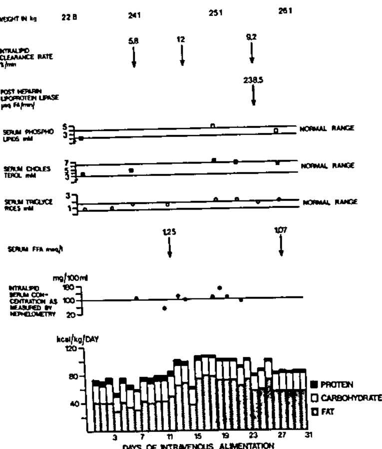


Fig 3 Total intravenous alimentation of a boy of 8 years with encephalitis

Vitamins and minerals were added in adequate amounts (1?) All solutions were infused in a peripheral vein of the child

Laboratory methods

The intravenous fat tolerance test (IVFTT) was carried out as described by Carlson & Rossner (3) For all tests a 20% Intralipid emulsion was administered intravenously in a dose of 0 1 g fat/kg body weight within one minute Capillary blood samples were collected every 5 minutes for 30 minutes The Intralipid concentrations were determined by nephelometry For each disappearance curve a minimum of four consecutive points was used The logarithms of the Intralipid concentrations were plotted against time Straight lines were obtained from which the correlation coefficients were calculated Steady state plasma Intralipid concentrations were estimated by nephelometry These levels were slightly overestimated no allowance being made for the fasting plasma nephelometric value which was unknown during the infusion of fat emulsion

The postheparin LPL activities were measured 5 minutes after the intravenous injection of 100 U heparin/kg body weight LPL activities were measured according to the method of Kelly (13) with minor modifications (8)

Serum triglycerides concentrations were determined *ad modum* Schmidt & von Dahl (??) Serum cholesterol concentrations were determined *ad modum* Carr & Drekter (4) Serum phospholipids concentrations were determined *ad modum* Zilversmit & Davis (?3) Lipoprotein electrophoresis was performed *ad modum* Postma & Stroes (17) For the determination of serum free fatty acids we used the extraction method described by Royer (?1) followed by titrimetric measurement according to Korovina (14) Serum ketone bodies were only qualitatively tested (Ketostix Ames)

RESULTS

The results of PN in patients 1–6 are shown in the corresponding Figs 1–6 Other data not shown in the figures are as follows

Liver function tests performed weekly remained normal in all patients Coagulopathy did not develop in any patient Qualitative examinations for the presence of ketone bodies in the blood were frequently performed in most patients with negative results irrespective the levels of blood lipids In patients 1 and 2 hyperlipaemia occurred associated with the

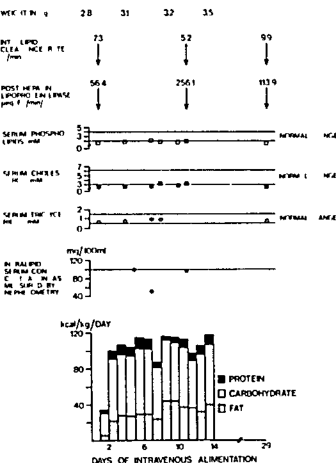


Fig 4 Total intravenous alimentation of a female infant of 22 days with intractable diarrhea

appearance of an increased pre β lipoprotein band on electropherograms In patient 3 hyper pre β lipoproteinaemia appeared only on the 20th and 21st day of the PN period In patients 5 and 6 hyperlipaemia did not occur even during high Intralipid doses In both patients 5 and 40 min postheparin LPL activities were measured the 40 min values were equal to or lower than the 5 min values

DISCUSSION

It can be seen from the results shown above that individual children widely differed in their tolerance for Intralipid During PN some patients (1 and 2) developed hyperlipaemia while receiving low quantities of fat whereas other patients (3, 4 5 and 6) tolerated very high fat doses and thus seemed to be very resistant to the development of hyperlipaemia Is it possible to predict and thus avoid the development of hyperlipaemia using as parameters either the postheparin LPL activity the IVFTT or the Intralipid blood levels?

The relation between fat tolerance and the postheparin LPL activity should be discussed first The postheparin LPL activity is of mixed origin as LPL has been found in extracts of many tissues such as adipose tissue muscle heart spleen lung kidney medulla and lactating mammary gland (19) Moreover a significant fraction of postheparin LPL seems to originate from the liver (15) The functional significance of postheparin LPL activity is therefore not exactly known It does however probably give an overall indication of the organism s capacity to eliminate triglyceride from the blood

Patient 1 in whom LPL was not determined and patient 2 in whom no rise of LPL was found tolerated only very small fat doses Patients 3 4 5 and 6 showed very high postheparin LPL activities during PN and tolerated very high fat doses Why did the postheparin LPL rise in some patients and not in others? To answer this question we looked for a correlation between the quantities of nutrients administered intravenously and maximal postheparin LPL activities We first calculated the means of the daily quantities of fat carbohydrate and protein given to the patients in the 3 days preceding the enzyme determinations No correlation could be found between the intake of either fat carbohydrate protein or a combination of two of these nutrients on the one hand and postheparin LPL activity on the other hand A good correlation coefficient (r=0 95 0 05<p<0 01) was found however between total caloric intake and postheparin LPL activity (Fig 7) As it is known that LPL decreases with starvation one could wonder whether the values at the start of the PN period could have been subnormal and have risen to normal during PN This is not the case since the initial values in our patients fall within the normal range found in our laboratory (82 8±32 4 n=4) and secondly because high LPL values during PN in patients 4 and 5

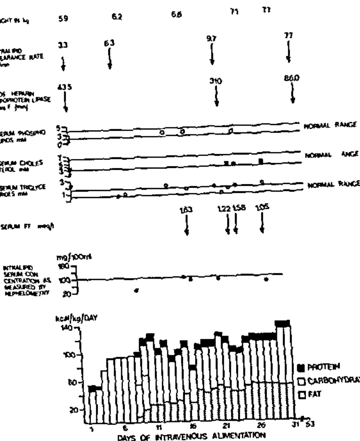


Fig. 5. Total intravenous alimentation of a girl of 13 months with intractable diarrhea.

decreased to the normal initial values after complete oral re-alimentation. A high caloric intake (probably accompanied by changes in insulin production) thus seems to induce synthesis of more enzyme. As no correlation could be found between fat intake and post-heparin LPL, it seems that the increased enzyme synthesis does not result from a specific fat inducement but is part of a more generalised anabolic effect of high-caloric PN.

The second question to be discussed concerns the relation between fat tolerance and the outcome of the IVFTT. With the exception of patient 6 who showed a threefold rise of fat elimination as measured by the IVFTT, all patients, while tolerating very different fat doses and some showing substantial rises in postheparin LPL activity, only showed slight deviations of IVFTT within the normal range for children (8.39±1.66, $n=10$). Thus the IVFTT did not give a good evaluation of the organism's total fat removal capacity. One reason for this could lie in the fact that the fat dose used in the IVFTT was too low. By giving such a low dose one probably only makes use of the small, directly available fraction of the total LPL pool that might be located on capillary endothelium receptors, as suggested by Robinson & Wing (18). This small fraction probably does not rise during PN. Support for the supposition that the IVFTT did not rise because of the low fat dose used in the test may be found in a recent study of Intralipid elimination in preterm and small for date babies (9). With a low fat dose (such as we used) Intralipid elimination curves were not significantly different in the two groups of patients. When a higher dose (0.5 g/kg body weight) was used

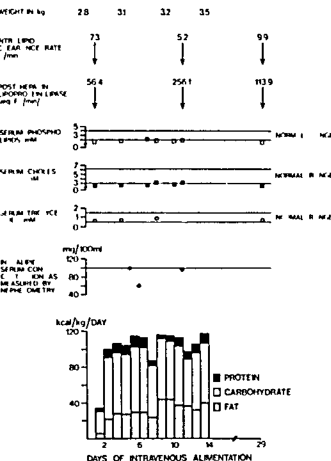


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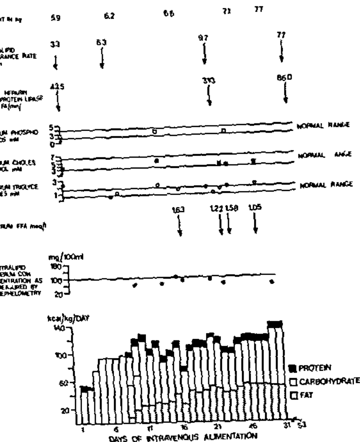


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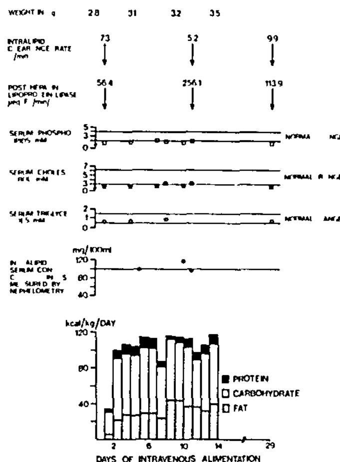


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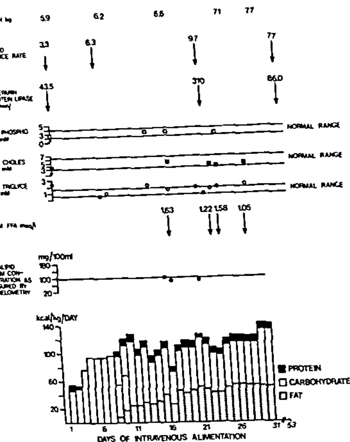


Fig 5 Total intravenous alimentation of a girl of 13 months with intractable diarrhea

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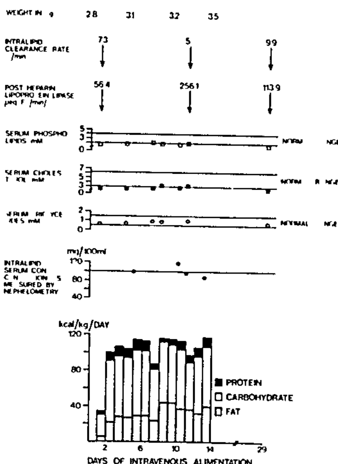


Fig 4 Total intravenous alimentation of a female infant of 22 days with intractable diarrhea

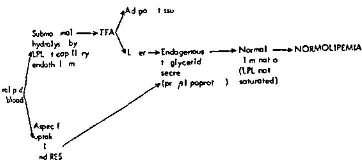
appearance of an increased pre β lipoprotein band on electropherograms In patient 3 hyper pre β lipoproteinaemia appeared only on the 20th and 21st day of the PN period In patients 5 and 6 hyperlipaemia did not occur even during high Intralipid doses In both patients 5 and 40 min postheparin LPL activities were measured the 40 min values were equal to or lower than the 5 min values

DISCUSSION

It can be seen from the results shown above that individual children widely differed in their tolerance for Intralipid During PN some patients (1 and 2) developed hyperlipaemia while receiving low quantities of fat whereas other patients (3, 4 5 and 6) tolerated very high fat doses and thus seemed to be very resistant to the development of hyperlipaemia Is it possible to predict and thus avoid the development of hyperlipaemia using as parameters either the postheparin LPL activity the IVFTT or the Intralipid blood levels?

The relation between fat tolerance and the postheparin LPL activity should be discussed first The postheparin LPL activity is of mixed origin as LPL has been found in extracts of many tissues such as adipose tissue muscle heart spleen lung kidney medulla and lactating mammary gland (19) Moreover a significant fraction of postheparin LPL seems to originate from the liver (15) The functional significance of postheparin LPL activity is therefore not exactly known It does however probably give an overall indication of the organism s capacity to eliminate triglyceride from the blood

Patient 1 in whom LPL was not determined and patient 2 in whom no rise of LPL was found tolerated only very small fat doses Patients 3 4 5 and 6 showed very high postheparin LPL activities during PN and tolerated very high fat doses Why did the postheparin LPL rise in some patients and not in others? To answer this question we looked for a correlation between the quantities of nutrients administered intravenously and maximal postheparin LPL activities We first calculated the means of the daily quantities of fat carbohydrate and protein given to the patients in the 3 days preceding the enzyme determinations No correlation could be found between the intake of either fat carbohydrate protein or a combination of two of these nutrients on the one hand and postheparin LPL activity on the other hand A good correlation coefficient ($r=0\ 95$ $0\ 05<p<0\ 01$) was found however between total caloric intake and postheparin LPL activity (Fig 7) As it is known that LPL decreases with starvation one could wonder whether the values at the start of the PN period could have been subnormal and have risen to normal during PN This is not the case since the initial values in our patients fall within the normal range found in our laboratory ($82\ 8\pm32\ 4$ $n=4$) and secondly because high LPL values during PN in patients 4 and 5



ig 8 Hypothetical model for Intralipid metabolism during PN when LPL is not saturated

back into the blood as pre β lipoprotein LPL being unsaturated the latter is normally eliminated and no hyperlipaemia ensues The second elimination mechanism involves direct aspecific uptake of fat in RES (5) and liver (20) Direct liver uptake probably plays only a minor physiological role as it has been demonstrated that there is very little net uptake of intact chylomicrons by the liver in rats (6) Here again fat is transformed by the liver into pre β lipoprotein which is secreted into the blood and normally eliminated because LPL is unsaturated

Fat elimination when saturation of LPL occurs is depicted in Fig 9 Surplus fat exceeding the LPL related removal mechanism is slowly eliminated by the second aspecific mechanism for fat removal Endogenous triglyceride secreted by the liver cannot be efficiently eliminated LPL being saturated Hyper pre β lipoproteinaemia develops accompanied by a rise of its three lipid components viz triglycerides cholesterol and phospholipids This model assumes the existence of a common saturable triglyceride removal mechanism for both exogenous and endogenous triglycerides Evidence for this may be found in a recent study of hyperlipaemic patients (2) The presence of high serum free fatty acid levels in patients 3 and 5 is in agreement with the hypothetical model It should be pointed out that ketosis did not occur The presence of elevated serum free fatty acid levels unaccompanied by ketosis can be ascribed to the fact that glucose was infused simultaneously

The following conclusions may be drawn from this study of Intralipid utilization in children First by controlling Intralipid blood levels daily and keeping these below 100 mg/100 ml during total PN one can adjust the Intralipid dose to the individual child s tolerance and thus take maximum advantage of the

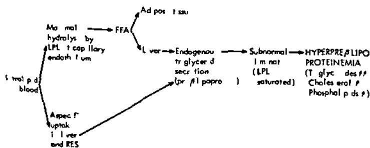


Fig 9 Hypothetical model for Intralipid metabolism during PN when LPL is saturated

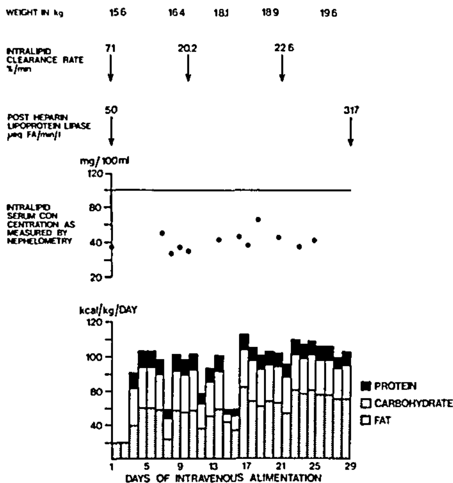


Fig 6 Total intravenous alimentation of a girl of 8 years with anorexia nervosa

small for date babies showed a decreased fat tolerance as compared with the preterm infants

The last question to be discussed concerns the relation between fat tolerance and the Intralipid blood levels In our patients hyperlipaemia developed whenever Intralipid blood levels exceeded a value of about 100 mg/100 ml This level corresponds with the LPL saturation level as described by Hallberg (10) It is noteworthy that the hyperlipaemia which occasionally developed was not limited to triglycerides but involved cholesterol and phospholipids too At the same time hyper pre β lipoproteinaemia could be demonstrated on electropherograms This can be explained by our hypothetical model of fat elimination during PN

Fat elimination when no saturation of the LPL system occurs is depicted in Fig 8 Circulating Intralipid can be eliminated in two different ways First, by the well known physiological removal mechanism involving the LPL enzyme system probably located in the capillary endothelium of peripheral tissues (19) This enzyme splits triglyceride molecules into glycerol and free fatty acids Most of the fatty acids that are liberated are deposited in adipose tissue after local resynthesis into triglycerides The rest remains in the blood stream is taken up by the liver and secreted

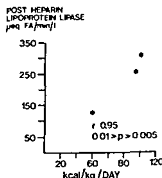


Fig 7 Correlation between the (highest) 5 minutes post heparin LPL activity and total caloric intake of 5 patients during PN

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PHOSPHORUS DEPLETION IN CHILDREN ON LONG TERM TOTAL PARENTERAL NUTRITION[1]

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ABSTRACT Ricour C Millot M and Balsan S (Laboratoire des tissus calcifies and Unite de Recherche sur les Maladies du Metabolisme chez l Enfant Hopital des Enfants Malades Paris France) Phosphorus depletion in children on long term total parenteral nutrition Acta Paediatr Scand 64 385 1975.—The retention of nitrogen calcium and phosphorus was studied in nine infants on total parenteral nutrition The amounts of calcium nitrogen and phosphorus were varied singly or simultaneously The results demonstrate close interrelationships in the retention of these three elements Not only the absolute amount of phosphorus perfused daily but also the amounts of nitrogen and/or calcium perfused simultaneously account for the phosphorus depletion that may lead to severe hypophosphatemia The decrease in serum phosphorus concentration with a simultaneous fall of urinary phosphorus excretion to undetectable levels and a rise in urinary calcium output to 10 mg/kg/24 hours or more are warning symptoms of phosphorus depletion Such a complication was observed in our first seven children on total parenteral nutrition Phosphorus depletion can be prevented by using the following amounts of these elements in the perfusate per 100 Kcal/kg/24 hours 400 mg/kg/24 hours of nitrogen 35 mg/kg/24 hours of calcium and 40 mg/kg/24 hours of phosphorus With such a technique no phosphorus depletion was observed in any of the 63 subsequent patients whom we treated with total parenteral nutrition for periods varying from 20 days to 9 months.

KEY WORDS Total parenteral nutrition phosphorus depletion calcium nitrogen

Since 1968 total parenteral nutrition has been used in several pathological states in adults (1 14 19 24 25 27) and in infants or children (3 5 6 7 10 11 12 13 16 18 22 29) Recently Dudrick et al have summarized some of the metabolic disorders encountered in patients submitted to this type of alimentation the side effects so far analysed concern glucose aminoacid calcium phosphorus essential fatty acid metabolism and other abnormalities such as hypo- or hyperkalemia anemia bleeding hypervitaminosis A and elevation in serum SGOT SGPT and alkaline phosphatase (8)

Over the last 4 years we have used long term total parenteral nutrition in 70 severely ill children with an average duration of 45 days per child Excellent results were observed in most cases However 7 of our first 10 patients developed a dramatic fall in their serum *phosphorus* concentration while serum calcium remained normal Severe yet reversible neurological disorders were observed in one infant during the period of hypophosphatemia

The purpose of the present study is (1) to analyse the factors favouring phosphorus

[1] A preliminary report of this paper was made at the 9th international congress of nutrition Mexico September 1972 (21)

high caloric value of fat emulsions without the risk of inducing hyperlipemia Second as caloric restriction seems to be incompatible with a normal to high fat elimination capacity one should be very cautious in the use of fat emulsions in children who for whatever reason receive a limited caloric intake during PN

As a general rule it seems advisable to start PN with a low daily fat dose and relatively high carbohydrate and protein intake Then Intralipid blood levels being kept below 100 mg/100 ml by daily control fat doses can be raised progressively Subsequently the major ity of patients will develop an increasing post heparin LPL activity coupled with an increased tolerance for fat till in the end they tolerate daily fat doses of 4–6 g/kg body weight

ACKNOWLEDGEMENT

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REFERENCES

1 Borresen H C Coran A G & Knutrud O Metabolic results of parenteral feeding in neonatal surgery *Ann Surg 172* 291 1970
2 Brunzell J D Hazzard W R Porte D Jr & Bierman E L Evidence for a common saturable triglyceride removal mechanism for chylomicrons and very low density lipoprotein in man *J Clin Invest 52* 1578 1973
3 Carlson L A & Rossner S Methodological study of an intravenous fat tolerance test with intralipid emulsion *Scand J Clin Lab Invest 29* 271 1972
4 Carr J J & Drekter J J Simplified rapid technique for extraction and determination of serum cholesterol without saponification *Clin Chem 2* 353 1956
5 Di Luzio N R Reticuloendothelial involvement in lipid metabolism *Ann NY Acad Sci 88* 244 1960
6 Felts J M & Mayes P A Lack of uptake and oxidation of chylomicron triglyceride to carbon dioxide and ketone bodies by the perfused rat liver *Nature 4980* 195 1965
7 Forget P P F X Fernandes J & Haverkamp Begemann P Enhancement of fat elimination during intravenous feeding *Acta Paediatr Scand 63* 750 1974
8 — Triglyceride clearing in glycogen storage disease *Pediatr Res 8* 114 1974
9 Gustafson A Kjellmer I Olegård R & Victorin I Nutrition in low birth weight infants *Acta Paediatr Scand 61* 149 1972
10 Hallberg D Elimination of exogenous lipids from the bloodstream *Acta Physiol Scand 65* Suppl *254* 1965
11 Hallberg D Holm I Obel A L Schuberth O & Wretlind A Fat emulsions for complete intravenous nutrition *Postgrad Med J 43* 307 1967
12 Harris J T Intravenous feeding in infants *Arch Dis Child 46* 855 1971
13 Kelly Th F Rapid assay of labelled free fatty acids in a mixture of labelled lipids *J Lipid Res 9* 799 1968
14 Korovina N A K metodike opredelenia neesterifitsirovannykh zhirnykh kislot v syvorotk krovi dete rannego vozrasta *Lab Delo 8* 471 1966
15 Krauss R M Windmueller H G Levy R I & Fredrickson D S Selective measurement of two different triglyceride lipase activities in rat postheparin plasma *J Lipid Res 14* 286 1973
16 Paulsrud J R Pensler L Whitten Ch F Stewart Sh & Holman R T Essential fatty acid deficiency in infants induced by fat free intravenous feeding *Am J Clin Nutr 987* 904 1972
17 Postma T & Stroes J A P Lipid screening in clinical chemistry *Clin Chim Acta 22* 569 1968
18 Robinson D S & Wing D R Regulation of adipose tissue clearing factor lipase activity In *Adipose tissue regulation and metabolic functions* (ed B Jeanrenaud & D Hepp) Academic Press New York and London 1970
19 Robinson D S The clearing factor lipase and its action in the transport of fatty acids between the blood and the tissues *Adv Lipid Res 1* 133 1963
20 Roger J Tolerance clinique anatomique et biologique de l organisme aux perfusions d emulsions de lipides chez l enfant *Extr Ann Anesth Franc 7* 805 1966
21 Royer M E A submicromolar assay for non polar acids in plasma and depot fat *Ann Biochem 20* 705 1967
22 Schmidt F H & van Dahl K Zur Methode der enzymatischen Neutralfett Bestimmung in biologischem Material *Z Klin Chem Klin Biochem 6* 156 1968
23 Zilversmit D B & Davis A K Microdetermination of plasma phospholipids by trichloroacetic acid precipitation *J Lab Clin Med 35* 155 1950

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Analytical procedures

The following techniques were used nitrogen Kjeldahl micromethod calcium an automated complexometric titration technique (Marius Calcium titrator Amsterdam Netherlands) phosphorus the technique of Fiske & Subbarow (9) adapted to an autoanalyzer Technicon

RESULTS

(A) *Variation of one parameter* (Fig 1)

Nitrogen During four consecutive periods the daily amounts of calcium (37 mg/kg) and of phosphorus (25 mg/kg) in the perfusate were kept constant in Case 1 The amount of nitrogen was progressively increased from 240 mg to 480 mg/kg/24 hours This increase promoted an increased nitrogen retention while serum calcium concentration remained normal By contrast a steep fall in serum phosphorus concentration from 5 3 mg to 2 5 mg/100 ml was observed Throughout the study phosphorus concentrations in the urine samples of this patient were at undetectable levels urinary excretion of calcium increased from 12 to 19 mg/kg/24 hours The phosphorus balance was at equilibrium (+0 6 mg/kg/24 hours) in the first period of the study but became negative (−4 −5 4 −5 mg/kg/24 hours) as the amount of nitrogen in the perfusate was increased

Calcium The amount of calcium in the perfusate was increased from 22 to 37 mg/kg/24 hours for one infant (Fig 1 Case 2) the amounts of nitrogen and phosphorus being respectively 325 mg and 25 mg/kg/24 hours Serum calcium concentration remained normal Urinary output of calcium increased from 5 to 14 mg/kg/24 hours Nitrogen retention was slightly increased Phosphorus concentration in the serum decreased to 3 7 mg/200 ml and urinary excretion of phosphorus dropped to undetectable levels The phosphorus balance was positive during the control periods (+4 8 and +1 3 mg/kg/24 hours) and became negative at −3 3 mg/kg/24 hours when calcium in the perfusate was increased

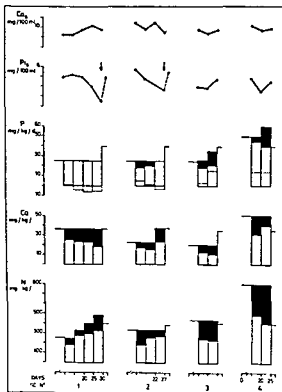


Fig 1 Total parenteral nutrition One variable In this figure variations in serum calcium (Ca s) and serum phosphorus (Pi s) are outlined The arrows indicate an addition of phosphorus in the solute after a drop in serum phosphorus Results of the study concerning retention of calcium phosphorus and nitrogen are represented by columns Each column shows a 5-day period with the top line indicating the intake the dark space showing urinary output The dotted lines represent theoretical phosphorus utilization In Case 1 only nitrogen intake was increased and in Case 2 only calcium was increased In Cases 3 and 4 phosphorus was the only variable

Phosphorus Phosphorus in the perfusate was increased by 10 mg per kg/24 hours in 2 patients (Fig 1 Cases 3 and 4) For one child the baseline amounts of calcium and phosphorus in the infused solutions were low 20 mg/kg/24 hours for calcium and 26 mg/kg/24 hours for phosphorus whereas in Case 4 all three elements were given in rather large quantities i e 800 mg/kg/24 hours for nitrogen 50 mg/kg/24 hours for calcium and 50 mg/kg/24 hours for phosphorus In both patients serum calcium concentration remained in the normal range Serum phosphorus concentrations increased

Table 1 *Cases studied*

Case no	Total parenteral nutrition indications	Sex	Age (months)	Initial body weight (grams)	Average weight increase (grams per day)
1	Protracted diarrhea severe malnutrition	M	6	4 250	30
2	Protracted diarrhea severe malnutrition	M	4	3 500	25
3	Hirschsprung s disease Ileostomia	M	7	4 325	20
4	Subtotal intestinal resection length of remaining intestine 25 cm	M	16	5 870	15
5	Protracted diarrhea severe malnutrition	F	13	6 500	20
6	Protracted diarrhea severe malnutrition	M	5	4 270	30
7	Subtotal intestinal resection length of remaining intestine 20 cm	M	4	3 125	30
8	Malrotation of the intestine short small intestine 60 cm protracted diarrhea	M	2	3 050	32
9	Fistula of the small intestine	M	3	2 950	30

depletion during long term total parenteral nutrition in children (2) to outline the daily requirements of parenteral phosphorus calcium and nitrogen that would possibly protect these patients from such accidents

MATERIAL AND METHODS

Subjects

Eight males and one female infant between 2 months and 16 months of age were studied Their weights at the beginning of this investigation varied between 2950 and 6500 g Total parenteral nutrition had been necessary because of the following pathological states subtotal resection of the small intestine ($n=3$) enterostomy ($n=2$) or chronic enteritis for which all attempts at enteral of peripherial parenteral nutrition had failed ($n=4$) (Table 1)

Technique of parenteral nutrition

Total parenteral nutrition was made possible with the use of an indwelling central venous catheter according to a method described previously (22)

Protocol of phosphorus calcium and nitrogen retention studies

During the whole study the amounts of glucose calories water chloride sodium potassium and magnesium administered daily in the perfusate were kept constant On a body weight basis the amounts infused each day were as follows calories 100 Kcal/kg water 130 ml per kg glucose 24 g/kg chloride 3 mEq/kg sodium 3 mEq/kg potassium 5 mEq/Kkg magnesium 10 mg/kg Vitamins trace elements and essential fatty acids were added daily to the perfusate (22) The amounts of calcium phosphorus and nitrogen were the only three parameters that varied singly or simultaneously Nitrogen was provided as a mixture of synthetic amino acids (Vamin Vitrum Stockholm) calcium as calcium gluconate (Calcium gluconate 10% Sandoz Rueil Malmaison France) and phosphorus as neutral di potassium phosphate (Pharmacie Centrale des Hopitaux Paris France) The daily amounts of these elements were varied from 250 to 800 mg/kg for nitrogen from 25 to 60 mg/kg for calcium and from 10 to 60 mg/kg for phosphorus Nitrogen calcium and phosphorus retentions were studied 8 to 15 days after the initiation of total parenteral nutrition Data were collected during 2 to 5 consecutive periods each period lasting 5 days Serum calcium and phosphorus concentrations were measured the first and last day of each period the other biological parameters were systematically controlled (22)—plasma electrolytes blood acid-base status blood urea nitrogen blood glucose plasma magnesium serum transaminases hemoglobin concentration Urines were collected daily in calcium free plastic bags Urinary excretion of calcium phosphorus and nitrogen were expressed in mg/kg of body weight/24 hours Evaluation began during a steady state in children whose stools weighed 30 g/24 g or less where nitrogen concentration can be considered nil The *retentions* of nitrogen calcium and of phosphorus were thus calculated by subtracting the amounts excreted in the urine from the daily amounts in the perfusate of each of these elements

The coefficients of retention were calculated as follows

$$\frac{\text{Retention mg/kg/24 hours}}{\text{Amount perfused mg/kg/24 hours}} \times 100$$

The theoretical phosphorus utilization for each period was estimated according to Reifenstein et al (20) The equation proposed by these authors is

Theoretical phosphorus utilization =

$$\frac{\text{Nitrogen retention}}{14\ 7} + \frac{\text{Calcium retention}}{2\ 23}$$

in mg/kg/24 hours

The actual balance of phosphorus was estimated by subtracting the theoretical phosphorus utilization from the phosphorus retention

of this child and his serum phosphorus con centration decreased to 1 5 mg/100 ml when he was perfused with 10 mg/kg/24 hours of phosphorus 320 mg/kg/24 hours of nitrogen and 50 mg/kg/24 hours of calcium The uri nary excretion of calcium was remarkably high 34 mg/kg/24 hours Phosphorus bal ance was negative −10 8 mg/kg/24 hours (*b*) during the subsequent periods when nitrogen and phosphorus in the perfusate were increased urinary excretion of phos phorus remained almost nil serum phospho rus concentrations were equal to or less than 2 mg/100 ml and phosphorus balances re mained negative until the amount of phos phorus in the infused solution reached 52 mg/kg/24 hours Urinary excretion of cal cium decreased as nitrogen and phosphorus were augmented in the perfusate

Phosphorus and calcium The amount of nitrogen in the infusion was 430 mg/kg/ 24 hours during three periods for one patient (Case 7 Fig 2) Calcium was increased from 20 mg/kg/24 hours to 32 mg then to 46 mg/kg/24 hours phosphorus from 33 mg to 36 mg/kg/24 hours Serum phosphorus concentration was 4 8 mg/100 ml at the be

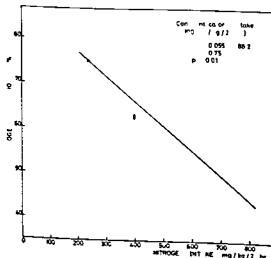


Fig 4 Total parenteral nutrition Negative correlation between nitrogen intake and the percentage of nitrogen retained while on a constant supply of calories (100 K cal/kg/24 hours)

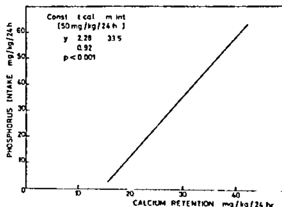


Fig 5 Total parenteral nutrition Positive correlation between calcium retention and phosphorus intake while on a constant calcium supply (50 mg/kg/24 hours)

ginning of the study and 4 0 mg/100 ml at the end Urinary excretion of phosphorus did not change Serum calcium concentration remained normal and urinary calcium excre tion increased from 9 mg to 14 mg/kg/ 24 hours Nitrogen retention decreased

Phosphorus Calcium and Nitrogen For 2 patients the amounts of all three elements were varied during the study (Fig 3) In both cases hypophosphatemia occurred and persisted when 10 to 30 mg/kg/24 hours of phosphorus in the perfusate were associated to high amounts of either calcium or cal cium and nitrogen At the same time phos phorus balances were found negative and urinary excretion of phosphorus remained at undetectable levels A rise of serum phos phorus concentration was promoted with the maintenance of a positive phosphorus bal ance (Fig 3 Case 9) when the amounts of phosphorus perfused were increased to 50 mg then to 62 mg/kg/24 hours Serum cal cium concentrations were found in the nor mal range calciuria decreased from 20 and 25 mg to 10 mg/kg/24 hours

(C) *Correlative studies*

Attempts to correlate retention rates and ex cretion rates of phosphorus calcium and nitrogen with the various amounts of the ele ments infused have shown the following re sults (*a*) under conditions where the daily

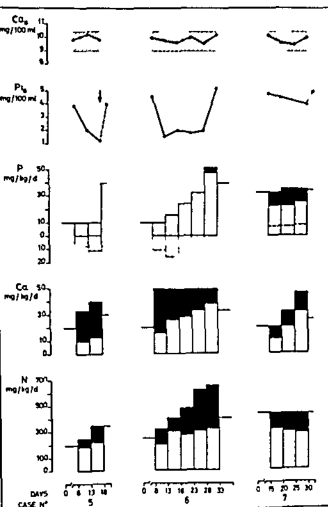


Fig 2 Total parenteral nutrition Same notations as in Fig 1 Simultaneous variations of two variables In Case 5 calcium and nitrogen are changed In Case 6 phosphorus and nitrogen are increased whereas in Case 7 phosphorus and calcium are increased

simultaneously with urinary phsophorus excretion The phosphorus balance was positive in the patient perfused with 26 mg/kg/24 hours of phosphorus The urinary excretion of calcium decreased from 19 to 10 mg/kg/24 hours in the second case

(B) *Simultaneous variation of two or three parameters* (Fig 2)

Nitrogen and Calcium In Case 5 the amount of phosphorus in the perfusate was kept constant and low at 10 mg/kg/24 hours nitrogen was increased from 250 to 350 mg/kg/24 hours and calcium from 33 to 45 mg/kg/24 hours Throughout the study phosphorus in the urine was undetectable Serum phosphorus concentration dropped from 3 8 to 1 2 mg/100 ml The negative phosphorus balance increased (from −7 4 to −10 8 mg/kg/24 hours) as nitrogen and calcium in the perfusate were increased Serum calcium concentrations remained normal and urinary excretion of calcium remained quite high (23 and 32 mg/kg/24 hours)

Phosphorus and nitrogen Patient no 6 was infused with 50 mg/kg per 24 hours of calcium while the amounts of nitrogen and phosphorus were increased simultaneously and progressively, in the former from 320 to 650 mg/kg/24 hours in the latter from 10 to 52 mg/kg/24 hours The following observations were made (*a*) there was no detectable phosphorus in the urine samples

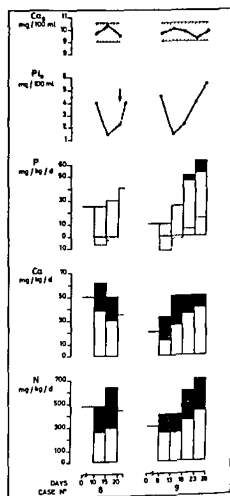


Fig 3 Total parenteral nutrition Same notations as in Fig 1 In Cases 8 and 9 simultaneous variations of three parameters calcium phosphorus and nitrogen

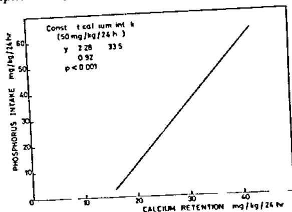


Fig 5 Total parenteral nutrition Positive correlation between calcium retention and phosphorus intake while on a constant calcium supply (50 mg/kg/'4 hours)

of this child and his serum phosphorus concentration decreased to 1 5 mg/100 ml when he was perfused with 10 mg/kg/24 hours of phosphorus 320 mg/kg/24 hours of nitrogen and 50 mg/kg/24 hours of calcium The urinary excretion of calcium was remarkably high 34 mg/kg/24 hours Phosphorus balance was negative −10 8 mg/kg/24 hours (*b*) during the subsequent periods when nitrogen and phosphorus in the perfusate were increased urinary excretion of phosphorus remained almost nil serum phosphorus concentrations were equal to or less than 2 mg/100 ml and phosphorus balances remained negative until the amount of phosphorus in the infused solution reached 52 mg/kg/24 hours Urinary excretion of calcium decreased as nitrogen and phosphorus were augmented in the perfusate

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(C) *Correlative studies*

Attempts to correlate retention rates and excretion rates of phosphorus calcium and nitrogen with the various amounts of the elements infused have shown the following results (*a*) under conditions where the daily

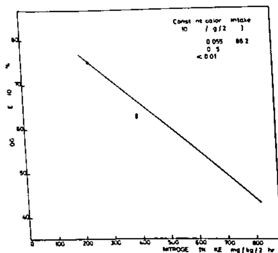


Fig 4 Total parenteral nutrition Negative correlation between nitrogen intake and the percentage of nitrogen retained while on a constant supply of calories (100 K cal/kg/'4 hours)

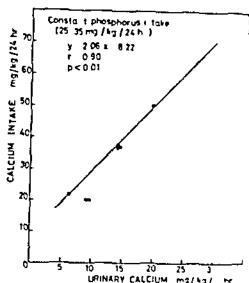


Fig 6 Total parenteral nutrition Positive correlation between calcium intake and urinary output while on a constant phosphorus supply (25–35 mg/kg/24 hours)

amounts of calories are kept constant there is a significant ($p<0\ 01$) negative correlation between the amounts of nitrogen infused and the coefficients of nitrogen retention (Fig 4) (*b*) when the amount of infused calcium is not varied a highly significant ($p<0\ 001$) positive correlation does appear between the amounts of phosphorus in the perfusate and calcium retention (Fig 5) (*c*) there is a positive correlation ($p<0\ 01$) between the amounts of calcium infused and calciuria when phosphorus in the infused solutions is kept at constant amounts (Fig 6)

DISCUSSION

During the present study plasma electrolytes blood acid–base status blood urea nitrogen blood glucose plasma magnesium serum transaminases hemoglobin concentration were found normal in our patients A decrease of serum phosphorus concentration was observed sometime during this investigation in all but one of the children (Fig 1 Case 3) The variations of serum phosphorus concentrations were more than 1 mg/100 ml and as much as 3 1 mg/100 ml with overt hypophosphatemia in 5 patients One patient had hypotonia areflexia lethargy tremor of extremities and polypnea These neurological disorders disappeared when normal serum phosphorus concentrations were maintained by increasing the amount of phosphorus in the perfusate (Fig 2 Case 5) Similar observations have been made in adults during total parenteral nutrition (15 17 23 26 28) and in one child with chronic renal failure on antacid therapy (2) In experimental phosphorus depletion a rise in serum calcium concentration simultaneous with the fall of serum phosphorus concentration has been reported (4) In our patients calcemia remained in the normal range throughout the study The biochemical findings that we regularly observed during the periods when hypophosphatemia occurred were (*a*) undetectable levels of urinary phosphorus (*b*) hypercalciuria 50 to 70% of the amount of calcium perfused being recovered in the urines of some patients (Fig 1 Case 1 Fig 2 Cases 5 and 6 Fig 3 Case 9) (*c*) negative phosphorus balances

The conditions favouring phosphorus depletion appear to be related (*a*) to the absolute amount of phosphorus perfused per kilogram of body weight per day (*b*) to the amounts of calcium and/or nitrogen associated to a given quantity of phosphorus in the perfused solutions

In fact all 3 children (Cases 5 6 and 9) receiving 10 or 16 mg/kg/24 hours of phosphorus with amounts of calcium and nitrogen varying from 33 mg to 50 mg and from 250 mg to 400 mg/kg/24 hours respectively developed a rapid and severe hypophosphatemia (Figs 2 and 3)

When the amount of phosphorus in the perfusate was more than 25 mg and less than 40 mg/kg/24 hours no change in serum phosphorus concentration was observed as long as the amount of calcium was less than 25 mg/kg/24 hours and that of nitrogen less than 450 mg/kg/24 hours (Fig 1 Case 3) On the other hand these same quantities of phosphorus became insufficient if they were

associated with higher amounts of calcium (Fig 1 Case 2) nitrogen (Fig 2 Case 7) or of both (Fig 1 Case 1 Fig 2 Case 6) Thus the interrelationship in the retentions of phosphorus calcium and nitrogen is such that the insufficiency of one of these elements in the perfusate may be responsible of the inappropriate utilization of the others Similarly an increased retention of calcium and/or nitrogen can be responsible for a negative phosphorus balance leading more or less rapidly to severe hypophosphatemia

The amount of phosphorus in the solution was greatly increased (50 to 64 mg/kg/24 hours) in 3 children In these patients (Cases 4 6 and 9) phosphorus balance was found to be positive (+9 mg/kg/24 hours to +15 mg/kg/24 hours) serum phosphorus concentration and calcium retention increased Yet the high concentration of phosphorus in the perfusate (34 4 to 50 mg/kg/100 ml) could not be maintained as salt precipitation occurred and blocked the Millipore filter

In the light of the above mentioned observations it appears that the following recommendations could be formulated in order to avoid phosphorus depletion and hypophosphatemia in children on long term total parenteral nutrition calories 100 Kcal/kg/24 hours nitrogen 400 mg/kg/24 hours calcium 35 mg/kg/24 hours and phosphorus 40 mg/kg/24 hours However it must be kept in mind especially for children with a severe malnutrition syndrome that total parenteral nutrition should never be initiated with solutions including more than 50 calories/kg/24 hours 160 mg/kg/24 hours of nitrogen 25 mg/kg/24 hours of calcium and 25 mg/kg/24 hours of phosphorus The composition of the perfusate must be altered gradually the increase in all elements being made simultaneously it is usually on the 10th day following the start of total parenteral nutrition that the above situation is reached Serum calcium and phosphorus concentration and urinary excretion of calcium and of phosphorus if checked weekly are valuable parameters Calciuria exceeding 10 mg/kg/24 hours and phosphaturia at undetectable levels should be considered as warning signs of a phosphorus depletion state By following these principles phosphorus depletion was not observed in any of the 63 other patients whom we had on total long term parenteral nutrition for the past 4 years

REFERENCES

1 Bittoun A Rambaud J C Loirat P Modigliani R Matuchansky C De Lagausie P Duhost C & Bernier J J Alimentation parenterale continue pendant 16 mois chez une femme ayant subi un arrachement traumatique de la totalite du jéjunoiléon *Arch Fr Mal App Dig 61* 39? 197?

2 Boelens P A Norwood W Kjellstrand C & Brown D M Hypophosphatemia with muscle weakness due to antacids and hemodialysis *Amer J Dis Child 120* 350 1970

3 Borresen H C Coran A G & Knutrud O Metabolic results of parenteral feeding in neonatal surgery *Ann Surg 172* 791 1970

4 Copp D H Parathormone calcitonin and calcium homeostasis *In* C L Comar & F Bronner (eds) *Mineral metabolism* vol 3 Academic Press New York 1969

5 Das J B Filler R M Rubin V G & Eraklis A J Intravenous dextrosen aminoacid feeding The metabolic response in the surgical neonate *J Pediatr Surg 5* 127 1970

6 Driscoll J M Heird W C Schullinger J N Gongaware R D & Winters R W Total intravenous alimentation in low birth weight *infants* a preliminary report *J Pediatr 81* 145 197_

7 Dudrick S J Wilmore D W Vars H M & Rhoad J E Long term total parenteral nutrition with growth development and positive nitrogen balance *Surgery 64* 134 1968

8 Dudrick S J MacFadyen B V Van Buren C T Ruberg R L & Maynard A T Parenteral hyperalimentation Metabolic problems and solutions *Ann Surg 176* 259 197?

9 Fiske C H & Subbarow Y The colorimetric determination of phosphorus *J Biol Chem 66* 375 1925

10 Filler R M Eraklis A J Rubin V G & Das J B Long term parenteral nutrition in infants *New Engl J Med 281* 589 1969

11 Ghadimi H Abaci F Kumar S & Rathi M Biochemical aspects of intravenous alimentation *Pediatrics 48* 955 1971

12 Harries J T Intravenous feeding in infants *Arch Dis Child 46* 855 1971

13 Heird W C & Driscoll J M Intravenous alimentation in pediatric patients *J Pediatr 80* 351 197?

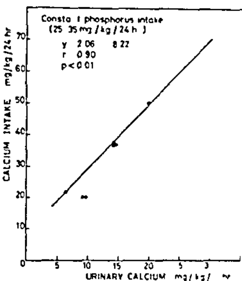


Fig 6 Total parenteral nutrition Positive correlation between calcium intake and urinary output while on a constant phosphorus supply (25–35 mg/kg/24 hours)

amounts of calories are kept constant there is a significant ($p<0\ 01$) negative correlation between the amounts of nitrogen infused and the coefficients of nitrogen retention (Fig 4) (*b*) when the amount of infused calcium is not varied a highly significant ($p<0\ 001$) positive correlation does appear between the amounts of phosphorus in the perfusate and calcium retention (Fig 5) (*c*) there is a positive correlation ($p<0\ 01$) between the amounts of calcium infused and calcium when phosphorus in the infused solutions is kept at constant amounts (Fig 6)

DISCUSSION

During the present study plasma electrolytes blood acid–base status blood urea nitrogen blood glucose plasma magnesium serum transaminases hemoglobin concentration were found normal in our patients A decrease of serum phosphorus concentration was observed sometime during this investigation in all but one of the children (Fig 1 Case 3) The variations of serum phosphorus concentrations were more than mg/100 ml and as much as 3 1 mg/100 ml with overt hypophosphatemia in 5 patients One patient had hypotonia areflexia lethargy tremor of extremities and polypnea These neurological disorders disappeared when normal serum phosphorus concentrations were maintained by increasing the amount of phosphorus in the perfusate (Fig 2 Case 5) Similar observations have been made in adults during total parenteral nutrition (15 17 23 26 28) and in one child with chronic renal failure on antacid therapy (2) In experimental phosphorus depletion a rise in serum calcium concentration simultaneous with the fall of serum phosphorus concentration has been reported (4) In our patients calcemia remained in the normal range throughout the study The biochemical findings that we regularly observed during the periods when hypophosphatemia occurred were (*a*) undetectable levels of urinary phosphorus (*b*) hypercalciuria 50 to 70% of the amount of calcium perfused being recovered in the urines of some patients (Fig 1 Case 1 Fig 2 Cases 5 and 6 Fig 3 Case 9) (*c*) negative phosphorus balances

The conditions favouring phosphorus depletion appear to be related (*a*) to the absolute amount of phosphorus perfused per kilogram of body weight per day (*b*) to the amounts of calcium and/or nitrogen associated to a given quantity of phosphorus in the perfused solutions

In fact all 3 children (Cases 5 6 and 9) receiving 10 or 16 mg/kg/24 hours of phosphorus with amounts of calcium and nitrogen varying from 33 mg to 50 mg and from 250 mg to 400 mg/kg/24 hours respectively developed a rapid and severe hypophosphatemia (Figs 2 and 3)

When the amount of phosphorus in the perfusate was more than 25 mg and less than 40 mg/kg/24 hours no change in serum phosphorus concentration was observed as long as the amount of calcium was less than 25 mg/kg/24 hours and that of nitrogen less than 450 mg/kg/24 hours (Fig 1 Case 3) On the other hand these same quantities of phosphorus became insufficient if they were

associated with higher amounts of calcium (Fig 1 Case 2) nitrogen (Fig 2 Case 7) or of both (Fig 1 Case 1 Fig 2 Case 6) Thus the interrelationship in the retentions of phosphorus calcium and nitrogen is such that the insufficiency of one of these elements in the perfusate may be responsible of the inappropriate utilization of the others Similarly an increased retention of calcium and/or nitrogen can be responsible for a negative phosphorus balance leading more or less rapidly to severe hypophosphatemia

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REFERENCES

1 Bittoun A Rambaud J C Loirat P Modigliani R Matuchansky C De Lagausie P Dubost C & Bernier J J Alimentation parentérale continue pendant 16 mois chez une femme ayant subi un arrachement traumatique de la totalité du jejunoiléon *Arch Fr Mal App Dig 61* 392 1972

2 Boelens P A Norwood W Kjellstrand C & Brown D M *Hypophosphatemia* with muscle weakness due to antacids and hemodialysis *Amer J Dis Child 120* 350 1970

3 Borresen H C Coran A G & Knutrud O Metabolic results of parenteral feeding in neonatal surgery *Ann Surg 172* 291 1970

4 Copp D H Parathormone calcitonin and calcium homeostasis *In* C L Comar & F Bronner (eds) *Mineral metabolism* vol 3 Academic Press New York 1969

5 Das J B Filler R M Rubin V G & Eraklis A J Intravenous dextrosen aminoacid feeding The metabolic response in the surgical neonate *J Pediatr Surg 5* 127 1970

6 Driscoll J M Heird W C Schullinger J N Gongaware R D & Winters R W Total intravenous alimentation in low birth weight infants a preliminary report *J Pediatr 81* 145 1972

7 Dudrick S J Wilmore D W Vars H M & Rhoad J E Long term total parenteral nutrition with growth development and positive nitrogen balance *Surgery 64* 134 1968

8 Dudrick S J MacFadyen B V Van Buren C T Ruberg R L & Maynard A T Parenteral hyperalimentation Metabolic problems and solutions *Ann Surg 176* 259 1972

9 Fiske C H & Subbarow Y The colorimetric determination of phosphorus *J Biol Chem 66* 375 1925

10 Filler R M Eraklis A J Rubin V G & Das J B Long term parenteral nutrition in infants *New Engl J Med 281* 589 1969

11 Ghadimi H Abaci F Kumar S & Rathi M Biochemical aspects of intravenous alimentation *Pediatrics 48* 955 1971

12 Harries J T Intravenous feeding in infants *Arch Dis Child 46* 855 1971

13 Heird W C & Driscoll J M Intravenous alimentation in pediatric patients *J Pediatr 80* 351 1972

14 Jeejeeboy K N Zohrab W J Langer B Phillips M J Kuksis A & Anderson G H Total parenteral nutrition at home for 23 months without complication and with good rehabilitation *Gastroenterology 65* 811 1973
15 Lichtman M A Miller D R Cohen J & Waterhouse C Reduced red cell glycolysis 2 3-Diphosphoglycerate and adenosine triphosphate concentration and increased hemoglobin-oxygen affinity caused by hypophosphatemia *Ann Intern Med 74* 562 1971
16 Lloyd Still J D Shwachman H & Filler R M Protracted diarrhea of infancy treated by intravenous alimentation *Am J Dis Child 125* 358 1973
17 Metzger R Burke P Thompson A Lordon R & Frimpter G W Hypophosphatemia and hypouricemia during hyperalimentation with an amino acid glucose preparation *J Clin Invest 50* 65 a 1971
18 Michener W M & Law D Parenteral nutrition the age of the catheter *Pediatr Clin N Am 17* 373 1970
19 Rea W J Wyrick W J McClelland R N & Webb W R Intravenous hyperosmolar alimentation *Arch Surg 100* 393 1970
20 Reifenstein E C Jr Albright F & Wells S I The accumulation interpretation and presentation of data pertaining to metabolic balances notably those of calcium phosphorus and nitrogen *J Clin Endocr 5* 367 1945
21 Ricour C & Balsan S Phosphorus in long term parenteral nutrition in infants *9th International Congress of Nutrition* p 145 (Mexico Abstract 1972)
22 Ricour C & Nihoul Fekete C Nutrition parentérale prolongée *Arch Franç Pediatr 30* 469 1973
23 Ruberg R Allen T MacFadyen B & Dudrick S J Hypophosphatemia during parenteral feeding *9th International Congress of Nutrition p 145* (*Mexico Abstract 1972*)
24 Scribner B H Cole J J Christofer G Vizzo J F Atkins R C & Blagg C R Long term total parenteral nutrition *JAMA 212* 457 1970
25 Shils M E Wright W L Turnbull A & Brescia F Long term parenteral nutrition through an external arteriovenous shunt *New Engl J Med 283* 341 1970
26 Silvis S F & Paragas P D Paresthesias weakness seizures and hypophosphatemia in patients receiving hyperalimentation *Gastroenterology 62* 513 1972
27 Solassol C Joyeux H Serron B Callis A Pujol H & Romieu C Long term total parenteral nutrition in man an artificial gut *Biol Gastroent 5* 591 c 1972
28 Travis S F Sugerman H J Ruberg R L Dudrick S J Delivoria Papadopoulos M Miller L D & Oski F A Alterations of red-cell glycolytic intermediates and oxygen transport as a consequence of hypophosphatemia in patients receiving intravenous hyperalimentation *New Engl J Med 285* 763 1971
29 Wilmore D W Groff D B Bishop H C & Dudrick S J Total parenteral nutrition in infants with catastrophic gastrointestinal anomalies *J Pediatr Surg 4* 181 1969

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DEVELOPMENT OF RENAL CONTROL OF SALT AND FLUID HOMEOSTASIS DURING THE FIRST YEAR OF LIFE

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ABSTRACT Aperia A Broberger O Thodenius K and Zetterstrom R (Department of Paediatrics Karolinska Institutet St Goran s Hospital for Children Stockholm Sweden) Development of renal control of salt and fluid homeostasis during the first year of life Acta Paediatr Scand 64 393 1975 —This study describes the development of renal control of salt and water homeostasis Twenty three infants aged 3 weeks to 13 months were studied with respect to glomerular filtration rate (GFR) *using single injection technique* ability to excrete an oral salt load ability to excrete water and diluting capacity GFR developed exponentially salt excretion linearly water excretion was unchanged and diluting capacity actually decreased A hypothesis is presented for the theoretical basis of this functional development taking into account the interdependence of the functional parameters studied This theory might well explain the high incidence of hypernatremic dehydration in infants

KEY WORDS Infants renal function glomerular filtration rate sodium excretion water excretion diluting capacity

It is well documented that renal function is low in relation to the size of the organism in most newborn mammals (11 19) In man glomerular filtration rate (GFR) related to body surface is generally considered to reach a relatively constant level at the age of 2 years (20) Little is known however about the pattern of the postnatal development of the various homeostatic functions of the kidney The homeostatic properties will of course depend on both glomerular and tubular function The question whether the balance between glomerular and tubular function is the same in the immature as in the mature kidney has been much discussed and is as yet not settled (9 11 19)

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The purpose of this study has been to follow the development of some homeostatic functions of the kidney namely salt and fluid excretion and diluting capacity during the first year of life in man Previous studies from this laboratory have described those functions in the neonatal period (4 5)

MATERIAL AND METHODS

Twenty three infants from 3 weeks to 13 months of age have been included in the study The ages of the infants are shown in Fig 1 All infants had normal deliveries and were of appropriate length and weight for gestational age according to Swedish standards (12) They were hospitalized for illnesses which had no influence on the general conditions and no one had any type of renal disease or other disorder that might affect water and electrolyte metabolism Arterial blood pressure recorded by sphygmomanometry was normal for age according to the standards given by Haggerty et al (15) All infants were normothermic and had no signs of intestinal malabsorption Before the study all infants re

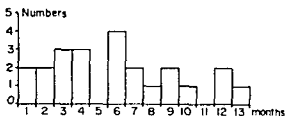


Fig 1 Number and age distribution of the infants studied

ceived a standardized food intake recommended by the Child Health Centers (BVC) (24)

A detailed history of food intake was obtained in each case No infant with special diet or abnormal salt intake was included During the first month after birth when the infants were fed only breast milk or cow milk s formula [1] they had a daily sodium intake of about 1 2 mEq/kg body weight Infants between 4 and 12 months of age were fed industrially prepared mixed food which should give a daily sodium intake of 5–6 mEq/kg and day (24) All studies were carried out in the nursery and the infants were kept in their own cots No signs of discomfort were noticed except in one 9 month old baby who vomited during the salt load This study was interrupted and is not included in the report

The following aspects of renal function were studied (*a*) The renal response to an oral salt and fluid load and (*b*) the glomerular filtration rate (GFR) In 12 infants only the salt load was performed and in one infant only the GFR was estimated In the remaining 10 infants both parameters were studied Informed parental consent was obtained in each case studied

Renal response to an oral salt load

At about 7 a m the infants received their ordinary formula appropriate for age At 9 to 10 a m the study was started by induction of water diuresis For this purpose the infants received breast milk or cow milk s formula (Baby Semp 1) diluted 1 3 with water in an amount of 2% of body weight during the first hour and thereafter by 0 5% of the body weight every half an hour during the entire study At least 1½ hours after the high fluid intake was started an oral salt load of 0 12 g sodium chloride per kg body weight (2 2 mEq Na^+/kg) was given

The sodium chloride was added to the diluted formula yielding a 1% NaCl solution It was generally administered by stomach tube In infants older than 8 months it was administered by a feeding bottle For that purpose the cow milk s formula had been diluted with salt free fruit juice instead of water Sodium chloride was then added yielding a 1% NaCl solution No ordinary food was given during the study Capillary blood samples were taken with heel puncture or fingerprick for determination of hematocrit serum concentration of sodium albumin and osmolality 30 minutes before and 120–240 minutes after the salt load

The glomerular filtration rate (GFR) was estimated as inulin clearance by following the disappearance curve after a single injection of this substance (10% inulin Laevosan Gesellschaft) For this purpose 0 1 ml blood was obtained in capillary samples 8–10 times during 80 minutes Details of this method have previously been described (4)

Analytical methods

The sodium concentration in serum and urine was analysed by flame photometer (Eppendorff) Inulin in blood was determined with the Anthron method (16) Osmolality in blood and urine was determined cryoscopically with the aid of a Knauer osmometer Serum albumin was determined refractrometrically Hematocrit was estimated in glass capillaries which were centrifuged at 10000 rpm for 5 minutes

Calculations

The average urinary sodium excretion (UNaV) per hour between 1–5 hours after the salt load was calculated Urinary water excretion (V) during high fluid intake was also calculated as mean value per hour The glomerular filtration rate (GFR) was calculated from the plasma disappearance curve of inulin according to the formula of Sapirstein (22) All values have been related to 1 73 m² body surface which has been regarded to be the most reliable reference when comparing renal function parameters during growth (20) Student s *t* test has been used in statistical analysis

RESULTS

To demonstrate the development of renal function from birth data have been included from 8 full term infants reported in previous studies (4 5) The development of the glomerular filtration rate (GFR) during the first year is demonstrated in Fig 2*a* and *b* All values are related to 1 73 m² body surface During the first 2 months after birth there is a steep increase in GFR The GFR then increases more slowly and towards the end of the first year reaches values found in older children (6) When the GFR is related to the logarithm of age (Fig 2*b*) the relationship is more linear

The renal response to an oral sodium load is shown in Fig 3*a* and *b* Fig 3*a* demonstrates the increase in the natriuretic response to the oral salt load Again the values are related to body surface The natriuresis is given as the average urinary sodium excretion (UNaV) per hour In contrast to the GFR the development of the natriuretic response is more linear During the first year UNaV increases about 10 times The values found at 10 to 13 months are in accordance with values earlier found in older children i e about 16 mEq/hour/1 73 m² (6)

[1] Baby Semp no 1 (Semper) sodium content 6 6 mEq/l Milkotal (Findus) sodium content 8 7 mEq/l human breast milk sodium content 7 mEq/l

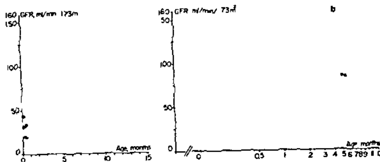


Fig 2 (*a*) Glomerular filtration rate (GFR) during first year of life The scale is lin–lin (*b*) Glomerular filtration rate (GFR) during first year of life related to the logarithm of age

The increase in the natriuretic response is out of proportion to the GFR as demonstrated in Fig 3*b*

The studies were carried out under standardized and maximal hydration sufficient to maintain a water diuresis throughout the study It thus seemed justified to compare the water excretion among the infants studied In contrast to GFR and the natriuretic responses urinary water excretion (V) is fairly stable during the first year of age This is demonstrated in Fig 4 It should however be noted that there are fairly large individual variations

Fig 5*a* and *b* demonstrate the diluting capacity in younger and older infants The diluting capacity is measured as free water clearance A characteristic relationship normally exists between the diluting capacity and the distal tubular sodium delivery which can be given as the sum of free water clearance (C_{H_2O}) and sodium clearance (C_{Na}) (1) Results obtained from our laboratory in 4 older children aged 7–12 years are included in the figure Newborn full term infants have a supernormal diluting capacity when compared with older children Fig 5*a* shows that the ability to dilute the urine remains supernormal during the first 5 months of life 5*b* demonstrates the diluting capacity in 6–13 months old infants The results from the older infants are more in accord with those found in older children

Serum concentrations of sodium and albumin and hematocrit did not change by the fluid and salt load and were also independent of age The mean sodium value was 136 mEq/l Serumalbumin 6 2 g/100 ml Hematocrit ranged from 31–53% mean value 38% The highest hematocrit values were found during the first month after birth the lowest values at 2–3 months of age

DISCUSSION

The developmental pattern of various parameters of renal function is very heterogeneous during the first year of life None of the homeostatic functions studied i e Na^+ excretion water excretion and diluting capacity follow the development of the GFR The GFR increases exponentially the ability to excrete Na^+ load increases linearly the ability to excrete water appears to have reached a level

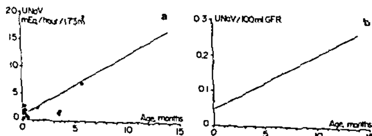


Fig 3 (*a*) The average hourly urinary sodium excretion (UNaV) during first year of life The correlation coefficient ($r=0.764$) is highly significant ($p<0.001$) (*b*) The development of the average hourly urinary sodium excretion (UNaV) in relationship to glomerular filtration rate The correlation coefficient ($r=0.755$) is significant ($0.01>p>0.001$)

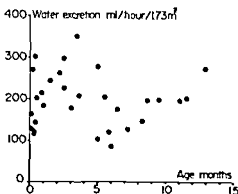


Fig 4 The average hourly water excretion during water diuresis in infants from birth to 13 months of age

already during the first month of life and the ability to dilute the filtered load actually decreases A hypothesis for the mechanism of development of these homeostatic functions taking in account their dependence on each other will now be presented

Since the anatomical developments of the glomerulus and the tubule do not appear to follow each other closely (13) it has often been suggested that the functional development of those structures will also proceed at different rates (11 13 19) The renal tubule is however composed of a series of different functional units the proximal tubule the descending loop of Henle (DLH) the medullary thin and thick ascending loop of Henle the distal tubule consisting of the diluting segment the distal convolution and the cortical and papillary part of the collecting duct The work carried out in each unit will depend not only on the functional capacity of the unit but also on the load which in turn depends on the filtration rate and the work in the preceding tubular units Thus the functional balance between various tubular segments is just as important as the balance between glomerular and tubular function for the homeostatic efficiency of the kidney The handling of Na^+ and water differs markedly in different parts of the tubule In the proximal tubule Na^+ is presumably reabsorbed both by active transport mechanisms and as a result of solvent drag (27) Water reabsorption follows Na^+ reabsorption and the Na^+ concentration of the tubular fluid does generally not change along the length of the proximal tubule (27) Pressure gradients between the peritubular capillaries interstitium and tubular lumen will to a large extent determine the Na^+ reabsorption (18) Those physical forces are among other things determined by the arterial blood pressure (3) and hematocrit (7 23) In DLH Na^+ transport out of and into the lumen is negligible (17) The tubular fluid is concentrated during the passage through DLH by a mostly urea-dependent osmotic withdrawal of water (17 21) and the addition of urea (21) It

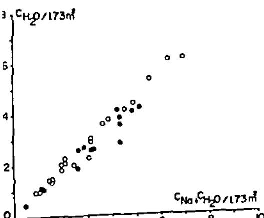


Fig 5 The relationship between free water clearance and the sum of sodium and free water clearance (distal tubular Na^+ delivery) during first year of life ○ newborn full term infants △ older children aged 7–12 years

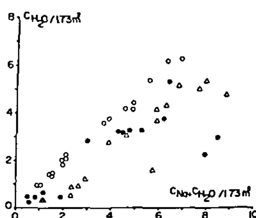


Fig 5 (*a*) ● Infants 3 weeks – 5 months of age (*b*) ● Infants 6–13 months of age

has been calculated that withdrawal of water will account for 60–70% of the concentration of fluid in the DLH (21) Withdrawal of water will result in the concentration of Na^+ creating a steep lumen interstitial Na^+ gradient (17) This gradient will promote the Na^+ reabsorption in the ascending loop of tubule and in turn create the optimal conditions for a very pronounced dilution of the urine If the osmotic concentration of the fluid in DLH were to be accomplished mainly by urea addition the concentration gradient of lumen interstitium Na^+ would be less steep and the net effect of Na^+ reabsorption in the ascending loop of Henle and the diluting segment would be less resulting in a less diluted urine under the condition of water diuresis It has recently been suggested from this laboratory that urea addition to the DLH will increase with increasing urea availability in the interstitium (2) The urea availability of the renal medulla in the newborn infant is most likely low and has been regarded as responsible for the low concentrating capacity of the newborn infant (10 26) In fact it has been shown that the concentrating capacity of the infant can be increased considerably with high protein intake (10) It is therefore suggested that the fluid concentration in the DLH in the immature kidney is accomplished by water withdrawal Thereby optimal conditions for Na^+ reabsorption in the ascending loop of Henle and the distal segment are created resulting in a high degree of urine dilution and Na^+ retention The hypothesis is supported by the observation in this study and in previous studies from this laboratory that in the very young infants which are unable to excrete an acute Na^+ load the diluting capacity is supernormal (4 5) In the older infants the diluting capacity as well as the ability to excrete a salt load appraoches normal adult values

Na reabsorption in the proximal tubule of the immature kidney is unpredictable as long as micropuncture data with identified puncture sites are not available The load to the proximal tubule is determined only by the filtration rate Anatomical observations suggest that the growth of the glomerulus precedes the growth of the proximal tubule in the first period of life (13) This would imply an inadequate reabsorption of the filtrate in this segment On the other hand the physical forces controlling proximal Na^+ reabsorption should by the low arterial blood pressure and the high hematocrit found in the newborn infant act to promote Na^+ reabsorption

To sum up the above presented hypothesis of the handling of Na^+ and water in the immature kidney proximal tubular Na^+ reabsorption could be low normal or in case of high hematocrit slightly increased The Na^+ reabsorption in the distal tubule is always enhanced This enhancement is reflected by the high diluting capacity It is most likely secondary to the low availability of urea in the renal interstitium Therefore the development of the ability to excrete a Na^+ load and the ability to concentrate urine will parallel each other

This hypothesis will explain the high incidence of hypertonic dehydration (14) in infants Due to the inability to concentrate the urine fluid cannot be retained adequately despite large losses The reabsorption of Na^+ is however unchanged and enhanced This will result in a more pronounced Na^+ than water retention which in itself will predispose to a hypertonic situation If in addition the diet is high in Na^+ the effect of the disproportionate urinary excretion of water and Na^+ will be potentiated It has also been shown that the incidence of hypertonic dehydration in infants on a high salt diet is increased (8 25)

REFERENCES

1 Aperia A Broberger O & Feychting H The effect of hypotonic mannitol and saline load on diluting capacity in man *Acta Physiol Scand 80* 145 1970

2 Aperia A Broberger O & Snellman K Tubular urea secretion during arginine infusion in dogs To be published

3 Aperia A Broberger O & Söderlund S Relationship between renal artery perfusion pressure and tubu

lar sodium reabsorption *Am J Physiol* 2?0 1205 1971

4 Aperia A Broberger O Thodenius K & Zetterstrom R Renal response to an oral sodium load in newborn full term infants *Acta Paediatr Scand* *61* 670 1972

5 — Developmental study of the renal response to an oral salt load in preterm infants *Acta Paediatr Scand* *63* 517 1974

6 Berg U Urine elimination of an oral salt and fluid load in healthy children *Acta Paediatr Scand* *62* 505 1973

7 Burke T J Robinson R R & Clapp J R Effect of arterial hematocrit on sodium reabsorption by the proximal tubule *Am J Physiol* *220* 1536 1971

8 Colle F Ayoub E & Raile R Hypertonic dehydration (hypernatremia) The role of feedings high in solutes *Pediatrics* *22* 5 1958

9 Edelmann C M Jr Pediatric Nephrology E Mead Johnson Award Address 1972 *Pediatrics* *51* 854 1973

10 Edelmann C M Jr Barnett H L & Troupkou V Renal concentrating mechanism in newborn infants Effect of dietary protein and water content role of urea and responsiveness to antidiuretic hormone *J Clin Invest* *39* 1062 1960

11 Edelmann C M Jr & Spitzer A The maturing kidney A modern view of well balanced infants with imbalanced nephrons *J Pediatr* *75* 509 1969

12 Engström L Karlberg P & Selstam U *Neonatal längdlykt i förhållande till graviditetstid* 1971 Copyright

13 Fettermann G H Shuplock N A Philipp F J & Gregg H S The growth and maturation of human glomeruli and proximal convolutions from term to adulthood Studies by microdissection *Pediatrics* *35* 601 1965

14 Finberg L Hypernatremic (hypertonic) dehydration in infants *N Engl J Med* *289* 196 1973

15 Haggerty R J Moroney M W & Nadas A S Essential hypertension in infancy and childhood *Am J Dis Child* 9? 535 1956

16 Hilger H H Klumper J D & Ullrich K J Wasser rucksresorption und Ionentransport durch die Sammelrohrzellen der Saugetierniere *Arch ges Physiol* *267* 218 1958

17 Kokko J P Membrane characteristics governing salt and water transport in the loop of Henle *Fed Proc* *33* 25 1974

18 Lewy J E & Windhager E E Peritubular control of proximal tubular fluid reabsorption in the rat kidney *Am J Physiol* *214* 943 1968

19 McCrory W W *Developmental Nephrology* Harvard University Press Cambridge Massachusetts USA 1972

20 — *Developmental Nephrology* p 90–108 Harvard University Cambridge Massachusetts USA 197?

21 Pennell J P Lacy F B & Jamison R L An in vivo study of the concentrating process in the descending limb of Henle s loop *Kidney Int* *5* 337 1974

22 Sapirstein L A Vidt D G Mandel M J & Hanusek G Volumes of distribution and clearances of intravenously injected creatinine in the dog *Am J Physiol* *181* 330 1955

23 Schrier R W & Earley L E Effects of hematocrit on renal hemodynamics and sodium excretion in hydropenic and volume expanded dogs *J Clin Invest* *49* 1656 1970

24 Socialstyrelsens medicinska expertgrupp för kost och fysisk aktivitet i barnaåldern 0–18 år (MEK B) *Socialstyrelsen redovisar* nr 33 1973

25 Taitz L S & Byers H D High calorie/Osmolar feeding and hypertonic dehydration *Arch Dis Child* *47* *257* *1972*

26 Winberg J Determination of renal concentrating capacity in infants and children without renal disease *Acta Paediatr Scand* *48* 318 1959

27 Windhager E E Some aspects of proximal tubular salt reabsorption *Fed Proc* *33* 21 1974

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POSTIRRADIATION SYNDROME AND EEG FINDINGS IN CHILDREN WITH ACUTE LYMPHOBLASTIC LEUKAEMIA

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ABSTRACT Garwicz S Aronson A S Elmqvist D and Landberg T (Departments of Paediatrics Clinical Neurophysiology Radiotherapy University Hospital Lund Sweden) Postirradiation syndrome and EEG findings in children with acute lymphoblastic leukaemia Acta Paediatr Scand 64 399 1975 —Out of 12 children with acute lymphoblastic leukaemia treated with craniospinal irradiation during primary haematologic remission 8 developed a postirradiation syndrome characterized by fever and tiredness The symptoms lasted 1–2 weeks and subsided spontaneously Longitudinal EEG studies revealed no acute disturbances during the irradiation therapy but in all cases studied moderate to severe diffuse general slowing developed during the postirradiation syndrome Complete normalization of the EEG occurred in all children at follow up It is concluded that the described EEG abnormalities constitute an integral part of the postirradiation syndrome

KEY WORDS Acute lymphoblastic leukaemia prophylactic CNS irradiation postirradiation syndrome diffuse generalized EEG abnormality

During the last few years an increasing number of children suffering from acute lymphoblastic leukaemia have been treated with prophylactic central nervous system (CNS) irradiation while in haematological remission (5 9) Recently a transient cerebral disturbance of somnolence and fever following cranial irradiation has been described (2 5 7) During this postirradiation syndrome EEG abnormalities were revealed in some patients (2 7) but their significance and specificity has been questioned (8)

In the present communication we would like to report on clinical features of the postirradiation syndrome and on EEG findings in serial examinations

PATIENTS AND TREATMENT

In 12 children aged –15 years with acute lymphoblastic leukaemia haematological remission had been induced with Vincristine and Prednisolone and maintenance therapy was given with 6-mercaptopurine In no case were there any signs of CNS leukaemia Cerebrospinal fluid was examined just before starting irradiation therapy and was found to be normal including cell cytology (1) in all patients At lumbar puncture Methotrexate was instilled intrathecally in a dose of 12 mg/m² body surface In the last 6 patients (Table I) Methotrexate was also given intrathecally at the end of the irradiation course and in patients R G B M and R P an additional dose of Methotrexate was given intrathecally during the radiotherapy

Prophylactic CNS irradiation therapy was performed during the primary haematological remission All patients received irradiation of the cranial and the spinal subdural space The neurocranium was irradiated with 2 opposed Co-fields at SSD 80 cm the viscerocranium being shielded by individually formed lead absorbers at the skin of the patients The central absorbed dose in the target in the cranium ranged between 2000 and 2500 rad given in 18–26 fractions The duration of therapy was 27–39 days except for patient R P who received a split-course treatment Correction for tissue heterogeneity has been done and the variation in absorbed dose in the target in the cranium was usually from +10% to −5% of the dose The spinal subdural space was irradiated usually with ⁶⁰Co through 2 3 or 4 adjoining posterior fields at SSD 50 cm the average absorbed dose in the spinal subdural space being 2300 rad given in 18 fractions during 30 days (median values)

Table 1 *EEG findings in 12 children with acute lymphoblastic leukaemia in relation to CNS irradiation and the occurrence of postirradiation syndrome*

Patient sex and age at diagnosis (years)	Duration of leuk before irrad (months)	Before irradiation (1-3 weeks)	During irradiation (weeks) 8 6 4 2 0	After irradiation (weeks) 2 4 6 8 10 12 14	Follow up (months after irrad)
PN ♂ 2	6 s			~	○ 28
MR ♀ 4	1	◔	◔ ○○○○○ ○	~ ○○	○ 6
NL ♂ 4	1	○	○ ○○○ ○	~ ○	○ 14
JH ♂ 4	8	○	○○○ ○○	○ ~	○ 14
CL ♀ 4	19	○	○○○	○ ~	○ 14
SÅ ♀ 4	22	◔	◔◔ ◑ ◑		○ 13
RG ♀15	1 s	○	○○	○	○ 10 †11
CF ♀ 6	1	○	○	~	○ 14
TM ♀ 2	2	○	○○○		†3
BM ♂15	1	◑	◑◑◑ ◑◑◑◑	◑◑◑ ●◑ ●●	†3
RP ♂ 2 s	1	○	○○ ○○ ○	○ ~ ○○ ○ ○ ○	○ 4
MK ♀ 3	1	◑	○ ○○○ ◔	◔ ~ ◔◔◔◔◔ ◔	○ 4

EEG code: ○ Normal borderline; ◔ Slight abnormality; ◑ Moderate severe abnormality; ● Very severe abnormality

⎵ irradiation ~ postirradiation syndrome

The subsequent maintenance chemotherapy consisted of 6 Mercaptopurine daily and Methotrexate and Cyclophosphamide once a week orally according to Pinkel et al (5) CNS relapse developed in patient B M 4 weeks after irradiation therapy and the patient expired 2 months later Another patient (T M) died 3 months after ^{60}Co therapy because of intercurrent infection Two patients (N L and R G) developed haematological relapse 10 and 9 months after the completion of irradiation therapy

METHODS

EEG examinations were performed 1–3 weeks before start of the irradiation therapy during and after the irradiation and at follow up The tracings were classified in respect to diffuse generalized abnormalities and graded into 4 groups (1) normal and borderline EEG (Fig 1C D F and I) (2) slight abnormality with a somewhat slow background activity and/or with *increase* in slow waves (Fig 1 A and G) (3) moderate–severe abnormality with a considerable general slowing with a great admixture of 1–3 Hz activity (Fig 1B E and H) (4) very severe abnormality with hardly any activity in frequencies above 3–4 Hz and high amplitude 1–2 Hz activity dominating the records In 3 patients several EEGs were analysed on line according to the method of normalized slope parameters (4) whereby mobility (a measure of the mean EEG frequency) was evaluated The analyses were performed on *bipolar* parieto-occipital leads over epoques of 10 seconds in duration and the mean of 12–20 continuous epoques was calculated for both hemispheres As no systematic side differences were observed the mean obtained from both sides has been plotted in Fig 2

Postirradiation Syndrome (PIS)

Eight of 12 children developed some 5–7 weeks after completion of irradiation therapy a complex of symptoms consisting of fever lassitude in some cases also anorexia and somnolence The symptoms lasted 1 or 2 weeks and subsided spontaneously There was no

Fig 1 Sequential EEG changes in 3 representative cases Recordings prior to the clinical symptoms (normal or slight abnormality) during the postirradiation syndrome (moderate–severe abnormality) and at follow-up (normal)

evidence of infection or neurological abnormalities In some patients bone marrow examination was performed during the PIS and was normal In the last 6 patients (Table 1) cerebrospinal fluid was examined just after the completion of irradiation therapy and showed normal protein content and total cell count as well as normal cell cytology In 3 patients lumbar tap was performed during the PIS In patient M R cerebrospinal fluid was normal

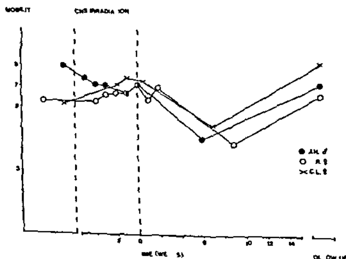


Fig 2 Mean EEG frequency in the 3 patients analysed Note the transient decrease of mobility during the postirradiation syndrome

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PN ♂ 2	6s		irradiation	~	O 28
MR ♀ 4	1	◔	◔ OOOOO	O ~ OO	O 6
NL ♂ 4	1	O	O OOO	O ~ O	O 14
JH ♂ 4	8	O	OOO OO	◔ ~	O 14
CL ♀ 4	19	O	OO◔	◑ ~	O 14
SÅ ♀ 4	22	◔	◔◔ ◑	◑	O 13
RG ♀ 15	1s	O	OO	O	O 10 †11
CF ♀ 6	1	O	O	~	O 14
TM ♀ 2	2	O	OOO		†3
BM ♂ 15	1	◑	◑◑◑ ◑◑◑◑	◑◑◑ ●◑ ●●	†3
RP ♂ 2s	1	O	OO OO O	◔ ~ ◔◔ O O O	O 4
MK ♀ 3	1	◑	O OOO	◔ ◔ ◑◑◑◑◑ ◔	O 4

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haematologic remission following systematic treatment A few patients needed however prophylactic cranial irradiation for normalization of EEG to occur Persistence of diffuse generalized EEG abnormality during irradiation therapy was in our material observed in one patient (B M) who soon developed overt CNS leukaemia and in another one (S Å) who 2 years earlier had had encephalitis with EEG changes Judged from our experience the irradiation had no immediate detectable effects on EEG only occasional EEGs were abnormal during the irradiation course The latent period until the occurrence of PIS comprised 5–7 weeks The clinical symptoms were always accompanied by EEG changes in the patients tested EEG abnormalities can however precede the clinical manifestations and persist longer than these Nevertheless in all patients the EEG was normal later at follow up This fact together with complete clinical recovery indicates that the PIS is a transient cerebral disturbance without longlived significance As such it should therefore not refrain the doctor from the further use of prophylactic CNS irradiation in the treatment of children with acute lymphoblastic leukaemia at least until better methods are available

ACKNOWLEDGEMENT

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ADDENDUM

Since the submittance of the paper another ? patients with acute lymphoblastic leukaemia were treated with prophylactic craniospinal irradiation Both of them developed the postirradiation syndrome with concomittant EEG changes identical to those described above

REFERENCES

1 Aronson A S Garwicz S & Somas R Cytology of the cerebrospinal fluid in children with acute lymphoblastic leukaemia *J Pediatr 85* 22? 1974
2 Freeman J E Johnston P G B & Voke J M Somnolence after prophylactic cranial irradiation in children with acute lymphoblastic leukaemia *Br Med J IV* 5?3 1973
3 Garcia J Buchwald N A Bach Rita G Feder B H & Koelling R A Electroencephalographic responses to ionizing radiation *Science 140* ?89 1963
4 Hjorth B EEG analysis based on time domaine properties *Electroenceph clin Neurophysiol 29* 306 1970
5 Hustu H O Aur R J A Verzosa M S Simone J V & Pinkel D Prevention of central nervous system leukaemia by irradiation *Cancer 32* 585 1973
6 Håkansson C H Lindgren M & Sulg I A EEG effects of postoperation irradiation treatment of brain tumours *Acta Radiol Ther Phys Biol 8* 301 1969
7 McIntosh S & Aspnes G T Encephalopathy following CNS prophylaxis in childhood lymphoblastic leukaemia *Pediatrics 52* 61? 1973
8 Pampiglione G Somnolence in children with acute leukaemia *Br Med J I* 158 1974
9 Report to the Medical Research Council by the Leukaemia Committee and the Working Party on Leukaemia in Childhood Treatment of acute lymphoblastic leukaemia effect of prophylactic therapy against central nervous system leukaemia *Br Med J II* 381 1973
10 Rubin P & Casarett G W *Clinical radiation pathology* W B Saunders Co Philadelphia London & Toronto 1968 vol II p 609
11 Sams C F Endo S Aird R B Adams G D & Ellman G L Functional response of the central nervous system of dogs to low level irradiation *Acta Radiol Ther Phys Biol 5* 177 1966

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but in patients R P and M K there were immature basophilic cells on cytologic examination without accompanying cell count increase Control examination 2 weeks later showed persistence of some blast like cells which disappeared some weeks later without therapy

EEG Findings

In Table 1 all EEG recordings are shown in relation to the irradiation therapy and the occurrence of PIS as described above Four patients showed a definite generalized EEG abnormality before start of irradiation therapy In 2 of them (M R and M K) EEGs became normal during the irradiation In 3 cases with normal pre irradiation records slight abnormalities occurred in an occasional record toward the end of the irradiation period Patients M R and N L had normal EEGs 1 5 respectively 2 5 weeks before the PIS but in patient M K the EEG was abnormal already 4 weeks before the manifestation of PIS (Fig 1)

Immediately before during and immediately after the occurrence of the PIS the EEGs showed generalized abnormalities in all 5 cases in whom EEG records were obtained at the appropriate time The EEG became normal in all these patients after the PIS subsided and the earliest normal record was obtained after 4 weeks (patient R P) In 1 patient (R G) a normal EEG was recorded 6 weeks after the end of irradiation and this patient showed no PIS Patient B M who developed CNS leukaemia showed very severe and progressive abnormalities in his EEG

At follow up 4–28 months after the end of irradiation therapy EEG was obtained in all 10 surviving patients and was normal in all

Fig 2 shows that the mobility (mean EEG frequency) analysed in 3 patients remained stable during the irradiation period It then decreased considerably between 5 and 10 weeks after the end of irradiation in all patients who had their PIS during this period At follow up the mobility had returned to the original level

DISCUSSION

The doses used in the prophylactic cranial irradiation in children with acute lymphoblastic leukaemia are well below the doses causing morphologic injury of the brain tissue (10) Experimental studies have however shown that even low doses of irradiation energy are able to produce functional changes in the brain as judged by electroencephalography (3 11) EEG changes were also observed in patients receiving radiotherapy because of brain tumours (6)

Recently a postirradiation syndrome (PIS) has been reported in children with acute leukaemia after prophylactic cranial irradiation (2) Its incidence varies from approximately 10% (5) to 78% (2) of treated patients and is apparently independent of concomitant intrathecal Methotrexate administration (2) Occasional EEG performed during the somnolent phase showed abnormal tracings improving after recovery (2 7) There are however no reports on longitudinal EEG studies in these patients and because of this the significance of EEG findings during the PIS has been questioned (8)

Our observations are in agreement with previous reports regarding symptomatology (fever and tiredness) incidence (about 66%) time of onset (5–7 weeks after completion of irradiation) duration (1–2 weeks) and the self limited nature of the PIS There are however several interesting points emerging from analysis of the longitudinal EEG recordings It is evident from our investigations that the majority of patients with acute lymphoblastic leukaemia have normal EEG after attaining haematologic remission before the start of prophylactic cranial irradiation This contrasts with the frequent occurrence of a patchy EEG abnormality observed by Pampiglione (8) on the day of diagnosis i e in the acute blastic phase of the disease One explanation of this apparent discrepancy may be that in the majority of patients initial foci of leukaemic involvement in the brain have disappeared at the time of

haematologic remission following systematic treatment A few patients needed however prophylactic cranial irradiation for normalization of EEG to occur Persistence of diffuse generalized EEG abnormality during irradiation therapy was in our material observed in one patient (B M) who soon developed overt CNS leukaemia and in another one (S Å) who 2 years earlier had had encephalitis with EEG changes Judged from our experience the irradiation had no immediate detectable effects on EEG only occasional EEGs were abnormal during the irradiation course The latent period until the occurrence of PIS comprised 5–7 weeks The clinical symptoms were always accompanied by EEG changes in the patients tested EEG abnormalities can however precede the clinical manifestations and persist longer than these Nevertheless in all patients the EEG was normal later at follow up This fact together with complete clinical recovery indicates that the PIS is a transient cerebral disturbance without longlived significance As such it should therefore not refrain the doctor from the further use of prophylactic CNS irradiation in the treatment of children with acute lymphoblastic leukaemia at least until better methods are available

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2 Freeman J E Johnston P G B & Voke J M Somnolence after prophylactic cranial irradiation in children with acute lymphoblastic leukaemia *Br Med J II* 5?3 1973
3 Garcia J Buchwald N A Bach Rita G Feder B H & Foelling R A *Electroencephalographic responses to ionizing radiation* *Science 140* 289 1963
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STUDIES ON MATURITY IN NEWBORN INFANTS

VII *Foetal Haemoglobin*

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ABSTRACT Finnstrom O, Gothefors L and Zetterlund B (Departments of Paediatrics University Hospitals Linkoping and Umeå Sweden) Studies on maturity in newborn infants VII Foetal haemoglobin Acta Paediatr Scand 64 404 1975 —Cord blood from 125 newborns of various gestational ages has been analysed for the ratio foetal haemoglobin to total haemoglobin using an alkali denaturation method The quotient percentage of foetal haemoglobin divided by birth weight correlates well with gestational age Thus the percentage of foetal haemoglobin in cord blood can be used as a method for estimating maturity in newborn infants Foetal haemoglobin has been compared with other methods for maturity assessments and seems to give the same precision in estimating gestational age as the best of these which is the scoring of external characteristics However the latter method is considerably less time-consuming and more suitable for routine use

KEY WORDS Maturity gestational age newborn infants foetal haemoglobin

We have previously in a series of papers discussed the difficulty of estimating maturity (or gestational age) in newborn infants The methods we used were anthropometric measurements—external characteristics neurological examination X ray of epiphyseal centres and motor conduction velocity (6 13 14 15 16) Although planned we were not able to include foetal haemoglobin (HbF) in these studies In 1955 Cottom (11) seems to have been the first to describe a correlation between gestational age and the percentage of foetal haemoglobin in cord blood In 1958 Brody (7) described foetal haemoglobin as a method of estimating maturity of newborn infants and further investigated the method in 1960 (8) He was rather optimistic as to the value of this method Later several other authors (1 2 5 12 19 20 21 22 25) have used foetal haemoglobin in the same situations as Brody but as a rule with less success We therefore thought it might be of value to perform a new study of this method comparing foetal haemoglobin with some of the methods we used earlier We also think that such comparison is

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Definitions and abbreviations used

Gestational age age in days from the first day of the mother s last menstrual period until the day of birth
40th week days 274 to 280
SGA = small for gestational age Infant with birth weight below −2 S D in the relation between birth weight and gestational age according to Swedish standard curves (24)
AGA = appropriate for gestational age Infant with birth weight within normal limits for the gestational age (between −2 and +2 S D)
LGA = large for gestational age Infant with birth weight above +2 S D according to Swedish standard curves
Pre term gestational age less than 267 days postmenstrual
Term gestational age between 267 and 294 days
Post term gestational age more than 294 days postmenstrual
Maturity as used by the authors maturity is an expression of the degree of development of the newborn infant Maturity thus defined is dependent on the gestational age of the infant but also on other factors such as biological variation

Table 1 *Important abnormal signs noticed during the perinatal period in the investigated material*

	Number
Low foetal heart rate during delivery	5
Postnatal asphyxia	
Apgar score below 7 at 1 minute	6
Apgar score below 7 at 10 minutes	1
IRDS	5
Neurological symptoms	3
Congenital heart defect	1
Isoimmunisation (mostly anti D)	10
Hyperbilirubinaemia (bilirubin above 15 mg/100 ml)	19

essential as it will otherwise be difficult to evaluate the method (17)

MATERIALS

Criteria for selection

Only infants for whom reliable information about gestational age was available were included in the study The criteria have been detailed earlier (13)

Investigated material

The material was collected during the period January 197? to April 1973 at the University Hospital of Umeå as a part of a joint obstetric and paediatric study dealing with various aspects of foetal and newborn maturity The material divided into gestational age groups of 2 week intervals is shown in Table 2 There were only 2 SGA infants 116 infants were AGA and were LGA 5 infants could not be classified according to Swedish standard curves which do not consider gestational ages below 33 weeks (2?5 days) 14 infants had slight to moderate dysmaturity signs (Clifford syndrome)

No mother had had any serious disease related to the pregnancy 7 mothers were diabetics and were treated with insulin throughout pregnancy 6 (1 twin mother) had had moderate toxemia (diastolic blood pressure above 90 mmHg in combination with albuminuria) 10 mothers had had minor bleeding at some time during pregnancy The frequencies of low foetal heart rate and low Apgar scores are seen in Table 1 as are also the most important abnormal signs during the neonatal period

METHODS

Birth weight was recorded as earlier described (13)

Eight external characteristics were examined and scored on the first or second day of life by one of us (B Z) using the technique earlier described (14) The interscoring reliability between two examiners was studied on ?0 infants The interscoring reliability was good the difference amounting to a maximum of 2 points in 3 infants 1 point in 8 infants and no difference in the remaining 9 infants

Neurological examination and scoring of 10 neurological signs was performed by one of us (O F) using the technique earlier described (15) Most infants were examined on day 5 a few on day 4 or 5 respectively

Foetal haemoglobin A heparinized blood sample was collected from the umbilical cord immediately after clamping and stored in a refrigerator until analysed The analysis was performed on day 1 or 2 in 109 cases on day 3 in 8 cases on day 4 in 5 cases and on day 7 in 3 cases Foetal haemoglobin was measured and calculated according to Brody s modification (7) of Betke s method (4) with some further modification as follows

0 1 ml of blood was first haemolysed in 200 ml of distilled water which contained 0 04% NH OH The rate of alkali denaturation was determined in a Guilford spectrophotometer type 300-N at 415 nm 6 0 ml of blood hemolysate was added to the cell and the extension read (E_0) The sample was continuously kept in water bath at 37°C Next 0 ? ml of 2 N NaOH was quickly pipetted into the cell the content was mixed and the extinction value (E_t) read at 30 sec intervals for 10 min The end point of denaturation was measured after about 1 hr The percentage of normal haemoglobin at time t(Hb_t) was calculated according to the formula given by Brody (8)

$$Hb_t = 100 \; \frac{E - E}{E_0 - E}$$

and correction was made for dilution by the alkali solution The relative amounts of foetal hemoglobin were assessed according to Betke (4) by plotting the logarithms of the Hb values against time and extrapolating the linear part of the curve to zero time

Control of methods Five samples of cord blood were stored for 1 week in a refrigerator at approximately +4°C All these samples were analysed for foetal haemoglobin after 1 3 and 7 days storage No significant changes in the relative amounts of foetal haemoglobin were noted during this time It was thus considered of minor importance on which day during the neonatal week the samples were analysed provided they had been stored in a refrigerator It was noted however that the sample was more difficult to handle when stored more than 3 days due to a great increase in viscosity As a comparison it can be mentioned that if samples were stored at −?0°C there was a definite decrease in the relative amount of foetal haemoglobin even after 1 days storage

Ten samples were analysed according to Brody and also with our modified method meaning that the samples were kept in a water bath at 37°C during the alkali denaturation thus diminishing the time to complete denaturation from about 3 hours to 1 hour No differences in the results were noted with these two methods

RESULTS

The mean values and standard deviations for percentage foetal haemoglobin of total haemoglobin are shown in Table 2 the infants being divided into gestational groups of 2 week

Table 2 *Mean and S D for percentage foetal haemoglobin Infants divided into gestational age groups of 2 week intervals*

	<225 $N=5$	225–238 $N=10$	239–252 $N=13$	253–266 $N=29$	267–280 $N=35$	>280 $N=33$	All infants $N=125$
Mean	89 76	89 79	86 90	85 46	78 19	79 23	82 45
S D	1 72	2 88	2 48	3 56	5 57	6 49	6 49

intervals In Table 3 the mean values and standard deviations for the quotient percentage of foetal haemoglobin divided by birth weight (as suggested by Brody 7, 8) are shown the infants being divided in gestational age groups of 2 week intervals

The individual values for percentage foetal haemoglobin as well as the individual quotients plotted against gestational age show that the resulting correlations fit reasonably well into a line or one within the age limits studied Thus the linear regression line for the correlation between the two values for foetal haemoglobin and gestational age—as well as for the other parameters studied and gestational age—were calculated These correlation coefficients are given in Table 4

The best correlation coefficient was for the correlation between gestational age and external score slightly better than that for the correlation between the quotient percentage foetal haemoglobin/birth weight and gestational age

The linear regression equation for Y (gestational age) on X (percentage of foetal haemoglobin divided by birth weight) was

$$Y=315-1\ 74\ X$$

The HbF values for the 14 infants with dysmaturity signs were evenly distributed about the regression line the same was true for the 19 infants with hyperbilirubinaemia (bilirubin above 15 mg/100 ml) and the 10 infants with iso immunization There were four twin pairs in the study three non identical with only minor differences in their respective HbF value and one non identical twin pair with identical HbF values

DISCUSSION

Discussion of the method

Our slight modification of Brody s (7) method for foetal haemoglobin estimation does not affect the results It requires less time but is still time-consuming The storage of the blood in a refrigerator for some days after birth also does not affect the results Freezing the sample on the other hand rapidly lowers the relative amount of foetal haemoglobin Thus a cord blood sample taken at delivery can be used later in the neonatal week for maturity estimations provided the sample has been stored in a refrigerator and has not been frozen

Discussion of results

The available literature (1 2 3 5 7 8 12 19 21 22 25) provides conflicting opinions as regards the value of determining foetal haemoglobin in cord blood in order to estimate maturity in newborn infants These diverging

Table 3 *Mean and S D for percentage foetal haemoglobin divided by birth weight Infants divided into gestational age groups of 2 week intervals*

	<225 $N=5$	225–238 $N=10$	239–252 $N=13$	253–266 $N=29$	267–280 $N=35$	>280 $N=33$	All infants $N=125$
Mean	54 94	43 86	35 09	28 82	23 37	21 59	28 29
S D	9 25	7 71	8 11	4 82	3 46	2 98	9 81

Table 4 *Coefficients for the correlation between gestational age and the parameters for maturity estimations under study*

Birth weight	0 81
Foetal haemoglobin	−0 62
Foetal haemoglobin/birth weight	−0 85
External characteristics	0 87
Neurological tests	0 78

opinions mainly relate to different ways of expressing the value of foetal haemoglobin Brody showed (7 8) that the quotient percentage of foetal haemoglobin divided by birth weight was considerably better correlated to gestational age than was the percentage foetal haemoglobin alone as did Gupta et al later (20) Most other authors did not perform this division by birth weight Brody also showed that this HbF quotient correlated much better to gestational age than did birth weight again confirmed by Gupta et al (20) Kirschbaum on the other hand estimating foetal haemoglobin by columnar chromatography did not find this increase in precision by dividing with birth weight (21) Bard et al (2) compared estimation of foetal haemoglobin by columnar chromatography with the estimation of foetal haemoglobin synthesis as determined by incubation with C14 leucine He found a better precision in estimating gestational age using the second method He did not however calculate the HbF quotient

It thus seems essential to divide the percentage of foetal haemoglobin by the birth weight if this estimation is to be used for assessing maturity in newborn infants Most authors did not make any comparison with the estimation of gestational age from birth weight or other methods for assessing maturity We have earlier pointed out the importance of making comparison with other methods in order to evaluate a given method for maturity assessment In an earlier study comparing anthropometric measurements external characteristics neurological tests motor conduction velocity and X ray of epiphyseal centres (17) it was shown that external characteristics and neurological tests gave the best precision in estimating gestational age The precision was ±3 weeks The present results indicate that the HbF quotient gave about the same precision in estimating gestational age as did external characteristics

Measuring foetal haemoglobin is more time-consuming however than the scoring of external characteristics To use columnar chromatography would be still more tedious than the alkali denaturation method Thus for practical purposes the simple method based on external characteristics seems to be superior to the other methods so far described for maturity estimations In this series neurological tests correlated slightly less well to gestational age than either external characteristics or the HbF quotient The correlation between birth weight and gestational age was surprisingly good and of the same order as that for neurological tests However there were very few infants with retarded intra uterine growth in this material as compared with the earlier one studied by the author

Because few infants with intra uterine growth retardation were studied no conclusion can be drawn regarding the influence on the percentage of foetal haemoglobin of this complication Bard et al (2) however showed that the proportion of foetal haemoglobin being synthesized in SGA infants was greater than expected for gestational age Thus it is possible that the estimation of foetal haemoglobin in SGA infants can lead to an underestimation of gestational age Salzberger et al (23) however found identical values in dizygotic twins in spite of quite varying birth weight within some pairs Bromberg (9) noticed an increased percentage of foetal haemoglobin in cord blood in a group with chronic maternal hypoxia compared with a control group The gestational ages of these infants were not given and thus the groups are difficult to compare Garby et al (18) found increased synthesis of foetal haemoglobin in one SGA infant and in an infant to a toxemic mother In this study however

we did not find any changes in expected HbF values in infants of mothers with toxemia or in infants with dysmaturity signs (Clifford syndrome) Further Cook et al (10) did not find any increase of HbF in infants with the Clifford syndrome i e in probable placental insufficiency

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VIII Alpha foetoprotein and Albumin

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KEY WORDS Maturity gestational age alpha foetoprotein

Since the discovery of α foetoprotein in man by Bergstrand & Czar (2 3) the interest regarding this protein has mainly focused on the possibility of diagnosing liver carcinomas (increased serum levels) and the possibility of intra uterine diagnosis of anencephaly and open spinal cord defects mainly by detecting increased levels in amniotic fluid

Bergstrand & Czar noticed a decreasing level of α foetoprotein in foetuses with advancing gestational age With the use of immunological methods it is now possible to demonstrate small levels also in newborn pre term or full term infants Bergstrand et al 1972 (3) demonstrated a correlation between the levels of α foetoprotein and gestational age as well as between albumin total protein and gestational age which has been confirmed by other authors (1 11 12 14 15 17 18) Thus some previous studies suggest that the estimation of α foetoprotein in blood from newborn infants is an additional method of value for assessing newborn maturity although present opinions differ in this context

The aims of the present study were to test this possibility by comparing α foetoprotein measurements (as well as albumin measurements) with other methods for maturity assessments on the same group of newborn infants (9)

MATERIALS AND METHODS

For definitions and abbreviations used see Finnstrom et al 1974 (10) The material consists of 125 newborn infants of various gestational ages examined at the University Hospital of Umeå Details have been given in an earlier paper (10) *Birth weight* was recorded after birth as earlier described (6) *Eight external characteristics* were examined and scored on the first or second day of life by using the technique earlier described (7) *Neurological examination* and scoring of 10 neurological signs were performed by using the technique earlier described (8) *α-foetoprotein and albumin* Cord blood was obtained at delivery The cord sera were frozen at −20°C and stored at this temperature until analysed α foetoprotein and albumin were determined by electrophoresis in agarose gel containing

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Table 3 *Means and S D for α-foetoprotein divided by birth weight Infants divided into gestational age groups of 2 week intervals*

	<225	225–238	239–252	253–266	267–280	>280	All infants
Mean	0.280	0.189	0.111	0.049	0.027	0.021	0.062
S D	0.035	0.118	0.067	0.044	0.018	0.020	0.020

tional age does not depend on this factor since SGA infants and AGA infants of corresponding gestational age have the same levels of α foetoprotein (4 16 17) It was shown in the present study that the quotient α foetoprotein/birth weight correlates better to gestational age than does α foetoprotein alone Still the correlation is too low to make this measurement of any particular value in maturity assessments The α foetoprotein measurements take considerable time and cord blood is essential since the levels decline rapidly after birth even in pre term infants (12 13 18)

In this study we also included albumin since it is well known that the serum proteins increase with advancing gestational age (8) Albumin is not a precise indicator of gestational age however

There is still no simple chemical method available which is superior to the scoring of external characteristics in assessing newborn maturity

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This study was supported by grants from Riksföreningen mot cancer grant no 72 110 project 735 B73-01X and from Prenatalforskningsfonden The technical assistance of Mrs Marie Adler Mathofer is acknowledged

Table 4 *Coefficients for the correlation between gestational age and the parameters for maturity estimations under study*

Birth weight	0.81
α-foetoprotein	−0.69
α-foetoprotein/birth weight	−0.75
Albumin	0.56
External characteristics	0.87
Neurological score	0.78

REFERENCES

1 Anagnostakis D Alpha 1 fetoprotein and gestational age *J Pediatr 83* 1093 1974
2 Bergstrand C G & Czar B Demonstration of a new protein fraction in serum from the human fetus *Scand J Clin Lab Invest 8* 174 1956
3 — Paperelectrophoretic study of human fetal serum proteins with demonstration of a new protein fraction *Scand J Clin Lab Invest 9* 277 1957
4 Bergstrand C G Karlsson B W Lindberg T & Ekelund H α foetoprotein albumin and total protein in serum from preterm and term infants and small for gestational age infants *Acta Paediatr Scand 61* 128 1972
5 Brody S The intra-uterine age of the foetus at birth *Acta Obstet Gynecol Scand 37* 374 1958
6 Finnström O Studies on maturity in newborn infants I Birth weight crown-heel length head circumference and skull diameters in relation to gestational age *Acta Paediatr Scand 60* 685 1971
7 — Studies on maturity in newborn infants II External characteristics *Acta Paediatr Scand 61* 24 1972
8 — Studies on maturity in newborn infants III Neurological tests *Neuropaediatrie 3* 72 1971
9 — Studies on maturity in newborn infants VI Comparison between different methods for maturity estimations *Acta Paediatr Scand 61* 33 1972
10 Finnstrom O Gothefors L & Zetterlund B Studies on maturity in newborn infants VII Foetal haemoglobin *Acta Paediatr Scand 64* 404 1975
11 Gutzeit D Bader H M & Oehme J Die bedeutung des α_1 Fetoproteins für das Kindesalter *Monatsschr Kinderheilkd 122* 98 1974
12 Hyvarinen M Zelyzner P Oh W & Stiehm R Influence of gestational age on serum levels of alpha 1 fetoprotein IgG globulin and albumin in newborn infants *J Pediatr 82* 430 1973
13 Karlsson B W Bergstrand C G Ekelund H & Lindberg T Postnatal changes of alpha foetoprotein albumin and total protein in serum of man *Acta Paediatr Scand 61* 133 1972
14 Lardinois R Anagnostakis D Ortiz M A & Delisle M Human α fetoprotein during the last trimester of gestation *Clin Chim Acta 37* 81 1972
15 Laurell C B Quantitative estimation of proteins by electrophoresis in agarose gel containing antibodies *Anal Biochem 15* 45 1966
16 Marklein G & Rings M Quantitative immunologische Bestimmung des Alpha Fetoproteins in serum reifer und unreifer Neugeborener sowie im

Table 1 *Means and S D for α-foetoprotein (expressed as mg/ml) Infants divided into gestational age groups of 2 week intervals*

	<225 N=5	225–238 N=10	239–252 N=13	253–266 N=29	267–280 N=35	>280 N=33	All infants N=125
Mean	0 46	0 37	0 28	0 14	0 09	0 08	0 16
S D	0 08	0 21	0 14	0 11	0 06	0 08	0 15

antibodies according to Laurell (15) using specific antisera produced in rabbits as described previously in detail (13)

RESULTS

A measurable amount of α foetoprotein was detected in all cases but one The mean values and standard deviations for α foetoprotein and albumin is shown in Tables 1 and 2 the infants being divided in gestational age groups of 2 week intervals In Table 3 the mean values and standard deviations for the quotient α foetoprotein/birth weight as suggested for foetal haemoglobin by Brody (5) is shown with the same separations in gestational age groups

The linear regression line for the correlation between the values for α foetoprotein as well as for the other parameters studied and gestational age were calculated The correlation coefficients are given in Table 4

DISCUSSION

Several authors have noticed a correlation between gestational age and the level of α foetoprotein in cord blood (1 4 11 12 14 16 17 18) Opinions differ as to the value of measuring this protein in the assessment of maturity in newborn infants Norgaard (7) gave a very optimistic view as to the value of the method in this context while Anagnostakis (1) pointed to the great scatter in results at a certain gestational age In this study we compared α foetoprotein with other methods for maturity assessments previously studied by us The results indicate that α foetoprotein is of limited value The correlation to gestational age was considerably lower than for example external characteristics a method recommended by us for routine work in maturity estimations (9)

The different opinions as to the value of α foetoprotein measurements in assessing maturity are in our opinion due to the fact that earlier authors did not compare this method with other methods for maturity assessments except the recording of birth weight The degree of correlation between birth weight (or other anthropometric measurements) and gestational age depend to a great extent on the way of selecting infants for the study A low number of SGA infants lead to a high correlation with birth weight and an impression of good possibilities of estimating gestational age from birth weight alone A high number of SGA infants lead to a low correlation The degree of correlation between α foetoprotein and gesta

Table 2 *Means and S D for albumin (expressed as mg/ml) Infants divided into gestational age groups of 2 week intervals*

	<225 N=5	225–238 N=10	239–252 N=13	253–266 N=29	267–280 N=35	>280 N=33	All infants N=125
Mean	28 00	27 4	31 5	34 2	37 8	39 7	35 57
S D	7 38	4 14	7 31	6 90	5 24	6 11	7 19

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FOUR YEAR OLDS IN A NEW SUBURB THE NEED FOR MEDICAL AND SOCIAL CARE

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ABSTRACT Lagerkvist B Lauritzen S Olin P and Tengvald K (Department of Paediatrics Karolinska Institutet St Goran s Hospital for Children Stockholm Sweden) Four year-olds in a new suburb The need of medical and social care Acta Paediatr Scand 64 413 1975.—The standardized Swedish health examination of 4 year-olds was performed in a residential suburb of Stockholm with a high percentage of young families and immigrants Emphasis was placed on identifying children in need of pedagogic and psychological measures Psychological problems were identified by a structured interview with the parents and an examination of the child including developmental screening tests and the parents assessment of the child s behaviour at home We have placed more emphasis on the findings and evaluation at the examination than on interview data when arriving at a psychological diagnosis The somatic part of the examination revealed only minor problems confirming previous studies on four year-olds in Sweden Twelve percent of the 257 children were referred for further psychological investigation and 16% were recommended early admittance to preschool because of psychological problems Among the 46 immigrant children who did not have any Swedish parent more than 50% did not speak Swedish and were recommended early admittance to preschool In the whole material 41% needed or were already (17%) in preschool or day nurseries This indicates unfulfilled demands on such services within the community The mother s perception of her lack of social contacts in the neighbourhood correlated with the presence of psychological problems and the need for subsequent measures for her child

KEY WORDS Child preschool health child behaviour child development

Standardized health examinations for 4 year-olds have been used in Sweden since 1968 (22) to trace children with physical psychological or social problems Previous investigations (9 12 18) report prevalence data in accordance with similar international studies (3 13) detecting among somatic disorders mainly sight defects and dental decay The reports have placed little emphasis on the rationale of the programme as to what facilities are needed to remedy detected problems or the consequences of observed abnormalities for the future development of the child as discussed by Haggerty North and Wallace among others (7 16 19) The relationships between the social circumstances of the children and detected problems were discussed in one study in a predominantly academic Swedish town (11)

The nation wide survey of 7 year-olds in England Scotland and Wales showed marked variations in reading ability physical coordination and bladder control in different regions and social circumstances (15) The need of similar analysis in different communities is obvious The suburban areas are of particular relevance since urban expansion is to a large extent geared by economic factors creating cities with little regard to the need of children

Serum von Säuglingen und Kindern *Z Kinderheilkd 113* 327 1972
17 Norgaard Pedersen B α_1 Fetoprotein concentration in cord serum as a parameter for gestational age *Acta Paediatr Scand 62* 167 1973
18 Sananes R Martin F Nivelon J L Sizaret P Bourgeaux C Bordes M & Alison M Etude cinetique de l alpha 1 faetoproteine senque chez le nouveau né *Arch Fr Pediatr 30* 1043 1973

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able 2 *Selected social and demographic ata of 257 four year olds and their families*

here were 256 mothers and one single father

	No	Percent
amily structure	257	100
one parent	44	17
two parents	212	83
data lacking	1	
Number of children in the family	257	100
one	80	31
two	129	50
three	35	14
four to eight	12	5
data lacking	1	
Nationality of the mother	256	100
Swedish	191	75
Finnish	44	17
other	20	8
data lacking	1	
Nationality of both parents or the single parent	257	100
Swedish	176	69
Swedish+foreign	25	10
foreign	55	21
data lacking	1	
Working conditions for mothers	256	100
not employed	152	60
part-time	35	14
full time	68	6
data lacking	1	
Social class of mother	256	100
I upper class	23	9
II middle class	94	37
III working class	136	53
data lacking	3	1

of two points scored The interview with the parents was made by a pediatric nurse in the child s home and lasted about one hour

Somatic examination

The vision screening test using Snellen s hooks in rows and the cover test were performed by the nurse The cover test was rechecked by the pediatrician Physical examination was carried out by one of the two pediatricians in the team

Psychological screening

The first part was a developmental screening performed by the pediatrician and it included the following six items draw a man test counting three objects threading three to nine pearls on a string buttoning and unbuttoning three buttons cutting a corner with a pair of scissors and a speech assessment

The diagnosis of possible mental subnormality was used when the child was unable to eat and to dress himself (interview data) and/or did not pass *three or more* developmental tests after a good contact was established If the child refused to participate the diagnosis was used when the failure was confirmed by the parents experience of the child at home

The second part of the screening was an appraisal by the pediatrician of the child s general behaviour during the session divided into (*a*) contact ability demonstrated by the degree of shyness clinginess and anxiety and (*b*) concentration ability revealed by the degree of restlessness and inability to concentrate

Each item of the psychological screening was graded on a scale from zero to two Zero indicated that the test was passed or no abnormality noted One point indicated that the test was passed with difficulty or after more than one trial or when the child showed mild signs towards the extreme type of behaviour noted above Two points were given when the child did not pass the test or showed marked behavioural deviance

Our clinical view that a child had psychological problems and the measures we suggested were based on the results of the psychological interview and the examination weighed together with the opinion of the parent as to how the child functioned at home No measures were taken if the parents preferred to manage a problem themselves

Individual symptoms such as bedwetting thumb sucking dependency etc as disclosed by the interview were not included in this study since Klackenberg (11) has shown poor correlation between the presence *per se* of such symptoms and later emotional disorders He pointed out that the total symptom load might be a better indicator When relating psychological problems to social factors we have chosen to look at all psychological problems including mental subnormality and subsequent measures together

Evaluation of the relationship between clinical diagnosis interview data and psychological screening data

There were weak positive correlations between the interview scores and detected problems and with subsequent measures The highest correlation was reached when the *number of two point* behavioural questions was used as an indicator (Table 1) However the scores from the psychological screening yielded much stronger positive correlations with the problems and measures taken This indicates that we have placed more emphasis on the findings and evaluation of the examination than *on interview data* This points at a possible examination bias favouring false positive diagnosis in for example normal and shy 4-year-olds frightened of doctors We have tried to neutralize this bias as previously mentioned by checking the history *from the* parents on the child s behaviour *at home*

The main problem of over-diagnosis involves the mild emotional aberrations False positive findings were *only* analysed with respect to those children referred for further *psychological* examination Among those 30 children 28 were considered by the child psychologist to need further *help* A *follow-up* at 6 or 7 years of age will give a better picture of the exam*iner s bias* and the significance for the child s future of the problems found

Statistical methods

Data were processed by means of an IBM 360 computer programme (4) It allowed the calculation of the correlation factor Goodman-Kruskal s gamma γ (5) The statistical

Table 1 *Psychological screening and interview scores correlated to presence of psychological problems and to measures recommended*

The correlation is given as Goodman Kruskal s gamma (5) $N=257$

	Interview score		Psychological screening score		
				Behavioural	
	Developmental	Behavioural	Developmental	Contact ability	Concentration ability
Psychological problems present	0.29	0.48	0.56	0.76	0.76
Measures taken	0.32	0.48	0.46	0.80	0.60

Suburban life is also characterized by loose knit social networks (3) leading to few personal contacts with relatives and neighbours that might influence the development of the child Therefore a study of 4 year olds was performed in a suburb of Stockholm with the following main objectives

1 To implement the standardized health examination with emphasis on the detection of children in need of pedagogic and psychological measures
2 to survey the actions taken within the framework of existing medical and social services and suggest new ways to alleviate detected problems and
3 to relate psychological problems among the children to their social background

MATERIAL AND METHODS

The investigation was carried out during 1972 in a suburb of Stockholm erected between 1968 and 1970 This residential area contained 16000 inhabitants in December 1972 and is made up of apartment houses compactly grouped around mostly hard surfaced playgrounds well separated from traffic Mainly young families with predominantly pre school children live in the area The percentage of immigrants is high The income structures for different family types is equivalent to that of the same family types in the city of Stockholm (1)

Five Child Health Centers (CHC) cover the entire area The children born in 1968 who lived within the districts of three of the CHCs at any time between the age of 4 and 4.25 years were included They were extracted from the county and parish registers in addition to those children already known at the CHC The 295 children located were invited by letter and/or telephone Of these 255 underwent a complete examination and two children a part of the examination between the ages of 3.75 to 4.25 years Eleven children (4%) older than 4.25 years when examined were included in the somatic part only

Twenty seven children (9% of the 295 children) were not examined of these 14 (5% of the 295 children) refused to participate The 14 abstainers were well known to the CHC Most of them had been seen at the CHC during the year prior to the investigation and participated in the vision screening and audiometric test Eight of these children were considered to require further follow up for somatic or socio psychological problems when their charts at the CHC were reviewed Four children were not known at the CHC and were not reached in time to be included During 1972 26 4 year-olds moved to and 20 moved from the suburb Six of the newcomers were not examined nor were 3 children leaving the area

The resources available to manage detected problems were early admittance to preschool or day nursery care closer follow up at the CHC the child psychologist at the CHC the Child Guidance Clinic (CGC) and the Child Psychiatry Clinic The only additional resource available to children in this area as compared with other suburbs of Stockholm was an experimental nursery school for 3 to 5 year-olds where the parents as a rule the mothers participated as co leaders (6)

The examination of 4 year-olds was carried out according to the directions of the National Board of Health and Welfare (22) and consisted of a social and psychological interview vision and hearing screening blood and urine analysis physical examination and developmental screening tests

Interview

The structured social and psychological interview was modified and expanded to include questions covering the parents present and previous places of residence their present working conditions arrangement for child day care as well as questions geared at their social activities in the last respect using the same questions as a recent Swedish survey on living conditions (8) (The interview form may be obtained from the authors on request)

The psychological part of the interview consisted of five developmental and twenty two behavioural questions —each graded from zero to two where zero indicated normality For each child a total developmental and a behavioural score could be computed as well as the number

Table 2 Selected social and demographic data of 257 four year olds and their families

There were 256 mothers and one single father

	No	Percent
Family structure	257	100
one parent	44	17
two parents	212	83
data lacking	1	
Number of children in the family	57	100
one	80	31
two	179	50
three	35	14
four to eight	12	5
data lacking	1	
Nationality of the mother	256	100
Swedish	191	75
Finnish	44	17
other	20	8
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Nationality of both parents or the single parent	257	100
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The main problem of over-diagnosis involves the mild emotional aberrations. False positive findings were only analysed with respect to those children referred for further psychological examination. Among those 30 children 28 were considered by the child psychologist to need further help. A follow-up at 6 or 7 years of age will give a better picture of the examiner's bias and the significance for the child's future of the problems found.

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Table 4 *γ coefficients and χ^2 analysis of selected social indicators to psychological problems and subsequent recommended actions*

Parameter	Psychological problem present				Subsequent action taken			
	γ	χ^2	$p<$	df	γ	χ^2	$p<$	df
Family type								
two parents/one parent	0 34	4 53	0 05	1	0 36	5 07	0 05	1
Day care								
in family/at home	0 32	2 76	N S	1	0 33	2 63	N S	1
in family/day nursery	0 21	1 39	N S	1		1 19	N S	1
Position among siblings								
No 2 (N=79)/No 1 (N=144)	0 39	7 41	0 01	1	0 33	4 62	0 05	1
No 2 (N=79)/No 3-8 (N=32)	0 42	4 27	0 05	1	0 40	3 60	N S	1
Mother s nationality								
Swedish/Finnish	0 40	6 38	0 05	1	0 38	5 50	0 05	1
other nationality/Finnish	0 47	3 95	0 05	1	0 47	0 90	N S	1
Contacts with relatives and neighbours								
Many/some/none	0 3	4 69	N S	2	0 26	8 78	0 05	2

Psychological problems related to social factors

Using the interview data concerning the social circumstances of the families we found weak positive correlations between one parent families and psychological problems among the children (Table 4) We did not find any correlation between either the presence of psychological problems or subsequent measures and such crude social variables as the social class of the mother mother working or not working There was no association with the type of lease on the family s apartment or on which floor the apartment was located No clustering of children with problems was found in any part of the suburb

The percentages of problems or measures did not differ significantly between the children cared for at home in family day care or at day nurseries (Table 4) However among the children at day nurseries no one was referred for psychological investigation In fact the only measure taken was closer supervision at the CHC Either the problems among these children were less severe or the measures available were not geared towards their problems

Those 4 year-olds who were second sibling showed significantly fewer problems and were recommended fewer measures than those who were either first born or the third or younger child in the family (Table 4)

There was no difference between the frequency of problems among the 4 year olds of Swedish mothers and immigrant mothers with the exception of the Finnish children They

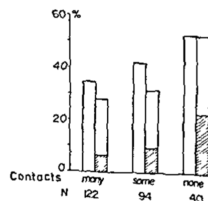


Fig 1 Psychological problems and subsequent measures related to the amount of the mother s contacts with relatives and neighbours according to the interview data Percentages are given for children with problems (left columns) and for measures taken (right columns) The hatched parts of the right columns represent the percentages of children who were referred for psychological investigation The figures below the columns denote the number of mothers with many few or no contacts

Table 3 *Behavioural and developmental problems known before 4 years of age detected at the examination and number of measures taken in 257 examined children*

Clinical diagnosis	Problems present: Before 4 years of age	At the examination	Total N	%	Measures taken: Follow up at the CHC	Recommended early nursery school by pediatrician	Referral to psychologist	Measures taken total N
Behavioural problem	5	70	75	29	26	18	21	65
Mental subnormality	5	21	26	10	9	4	9	22
Uncooperative at the examination	–	2	2		1	1	–	2
Total	10	93	103	39	36	23	30	89

methods used were chosen because the main part of the variables used in the psychological and social evaluation were of an ordinal type The gamma coefficient gives a +1 0 value for concordance 0 for independence and −1 0 for discordance χ^2 analysis for significance was used since other applicable significance tests for ordinal data are lacking (5)

RESULTS

Socio economic background

Some demographic characteristics of the 4 year olds and their families are presented in Table 2 The family structure in the suburban area under study did not differ from similar communities in urban Sweden (20) The percentage of immigrant families was high with a Finnish predominance Forty percent of the mothers were working outside their homes Among them 42 had their 4 year olds in day nurseries (17% of all 4 year olds) 37 (14%) in family day care and 24 (10%) had arranged for day care in their homes The distribution of the families in three socio economic classes according to occupation (8) did not differ from that of other new suburban apartment areas in Stockholm (1)

Psychological and social problems

The majority of the problems were detected in the psychological screening However such a problem in a child is not necessarily a sign of individual pathology but can be a symptom of disturbed relationships within the family or negative social influences on the whole family

Twenty nine percent of the children showed behavioural problems and 10% showed possible mental subnormality (Table 3) Twelve percent of the children were referred to the psychologist and could be considered to constitute a group of children with more serious psychological problems

Measures taken

Forty two children were recommended early admittance to nursery schools either by the pediatrician or the psychologist because of psychological problems Further measures taken with the 30 children referred to the psychologist were 19 children were recommended early admittance to nursery school 7 children were cared for by the CGC in the area and 4 children were referred for a full developmental test

Early admittance to nursery schools was also recommended for immigrant children who did not speak Swedish Of those 35 children whose parents or single parent were Finns 13 did not speak Swedish Among the 20 children with parents of other nationalities 12 did not speak Swedish Four of these children were already in day nurseries and the remaining 21 were recommended early preschool admittance Among the 212 children staying at home or in family day care during weekdays 63 were recommended early preschool training Thus in the whole material 106 of 255 4 year olds (41%) needed or were already in day care nursery or preschool care

Table 4 *γ coefficients and χ² analysis of selected social indicators to psychological problems and subsequent recommended actions*

Parameter	Psychological problem present				Subsequent action taken			
	γ	χ²	p<	df	γ	χ²	p<	df
Family type								
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Day care								
in family/at home	0 32	2 76	N S	1	0 33	2 63	N S	1
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No 2 (N=79)/No 3–8 (N=32)	0 42	4 27	0 05	1	0 40	3 60	N S	1
Mother s nationality								
Swedish/Finnish	0 40	6 38	0 05	1	0 38	5 50	0 05	1
other nationality/Finnish	0 42	3 95	0 05	1	0 42	0 90	N S	1
Contacts with relatives and neighbours								
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There was no difference between the frequency of problems among the 4 year olds of Swedish mothers and immigrant mothers with the exception of the Finnish children They

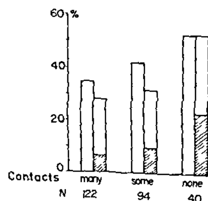


Fig 1 Psychological problems and subsequent measures related to the amount of the mother s contacts with relatives and neighbours according to the interview data Percentages are given for children with problems (left columns) and for measures taken (right columns) The hatched *parts of the* right columns represent the percentages of children who were referred for psychological investigation The figures below the columns denote the number of mothers with many few or no contacts

Table 5 *Number of somatic problems known before 4 years of age detected at the examination and number of referrals in 268 examined children*

Diagnostic category	Known before age 4 years	Detected at the examination	Total	Referrals
Strabismus or reduced visual acuity	17	20	37	24
Skin disorders	9	5	14	-
Adenoid tonsillitis otitis	7	6	13	7
Orthopedic problems	5	8	13	8
Inguinal hernia retentio testis	2	9	11	10
Asthma and allergic rhinitis	5	2	7	2
Neurological	3	1	4	1
Cardiovascular	1	3	4	3
Other or multiple problems	6	10	16	6
Total	55	64	119	61

showed significantly more problems than all other children They were also significantly more often recommended some measures than were children of Swedish mothers (Table 4) We disregard this difference since our tests might catch cultural differences

The mothers were also asked whether they considered themselves to have many some or no contact with neighbours or relatives in the area There were weak positive correlations between fewer contacts and such social background factors as single mothers (γ=0 17) and immigrant parents (γ=0 27) The number of contacts were independent of such factors as social class (γ=0 05) and if the mothers were working or not (γ=−0 10)

The mother s perception of her social contacts was related to detected psychological problems and subsequent actions (Fig 1 and Table 4) More problems were noted and more actions taken in children of mothers with fewer contacts This trend was significant for the measures recommended Furthermore all the children of mothers with no contacts were recommended some type of follow up or further evaluation indicating that the problems were more intractable or that these families could not cope with them without outside help

Somatic problems

The various somatic problems recognized at the examination are shown in Table 5 In general the more serious disorders were known and adequately dealt with before the 4 year-old control Among the newly diagnosed problems the majority concerned minor otological surgical and orthopedic problems which however required follow up or treatment by specialists The visual defects could be considered to be a major health problem Seventy five (28%) of the 268 children had unfilled carious teeth and 11 of these children (4%) were considered to have severe caries requiring emergency dental measures

DISCUSSION

One objective of this study was to identify 4 year olds with special needs for psychological or pedagogic measures using the screening procedure suggested for 4 year olds by the National Board of Health and Welfare (22) The percentage of children with psychological problems was high (Table 3) There are no data available from other studies of 4 year olds for comparison in this respect The number of referrals for psychological investigation corresponds with published data (9 18) This indicates that major psychological problems among the 4 year olds were not more frequent in the suburb under study than in other Swedish communities

A follow up study at 7 years of age may show to what extent our diagnoses and suggested measures have been valid or appropriate both regarding children identified as having problems and those considered problem free

Thirty percent of home and family day care children were judged to need priority preschool admittance In Sweden few data are available for comparison In one preliminary report (14) from a similar suburban area based on parent interviews but not on child observation 19%

of the children were recommended early admittance to preschool The different figures may partly be explained by differences in methodology The high percentage of preschool recommendations in the present study in part reflects the language problem encountered among children whose parents are immigrants The finding of a considerable need for preschool facilities for 4 year-olds has practical implications for the type of preschool that is required in some urban areas In Sweden a compulsory preschool for all 6 year-olds will be available within the next few years (21) In many suburban areas all 6-year olds and most 5 year-olds already attend the municipal preschools The findings indicate that more preschool facilities will be needed at least in newly erected suburbs to meet the demand disclosed by future 4 year-old examinations The improvement of the available resources within the community seems to be mandatory before further screening procedures are routinely introduced Furthermore the preschool organization and its aims need to be scrutinized taking into consideration the origin of the problems detected among the 4 year olds In our view there are several equally important factors interacting to make the child present psychological problems the child s temperament (17) the interaction between the parents and the child and his siblings and the interaction between the family and the society The child–parents interaction might be influenced in a preschool setting that also directly involves the parents Such a programme is presently under study in the area (6)

In the analysis of the correlation between the psychological problems detected among the 4 year-olds and their social environment we found no or only weak associations with crude indicators of the social status of the family This does not exclude that such associations do exist The characteristics of this suburb like other similar newly erected areas which are rapidly expanding with poor municipal transportation and few job opportunities might cause disturbances common for many residents irrespective of their social background Some indication of this possibility is suggested in the results concerning the social network of the families Those mothers who had no contacts with relatives or neighbours had 4 year-olds with more problems requiring more measures This is perhaps an important ingredient of urban pathology When the social network of the parent is weakened the child becomes more vulnerable or at least requires some type of external compensation

ACKNOWLEDGEMENT

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REFERENCES

1 Blixell U Bohman E Eriksson A M Erlandsson E Kadin U & Lindahl D *Tensta Befolknings struktur inkomststruktur och miljo* (Mimeograph) Socialhogskolan Stockholm 1973
2 Bott E *Family and social network* Tavistock London 1971
3 Davie R Butler N & Goldstein H *From birth to seven* Longman London 1972
4 Eldh B & Bonsdorffs I *Manual till korstabulerings programmet Table 3* Statens Institut för Byggnads forskning Stockholm 1971
5 Galtung J *Theory and methods of social research* Universitetsforlaget Oslo 1969
6 Grimfeldt U & Lauritzen S *Preliminar rapport over försoksverksamheten med utegrupper i Varberg* (Mimeograph) Stockholms Barnavårdsnamnd Stockholm 1971
7 Haggerty R J Research issues in child health II Some medical and economic issues *Pediatrics* 45 707 1970
8 Johansson S *Om levnadsnivaundersokningen Utkast till kapitel I och 2 i betankande om svenska folkets levnadsförhallanden* Allmanna Forlaget Stockholm 1970
9 Karlberg P & von Sydow G Goteborgsforsoket med hälsokontroll av fyraånngar 13 Sammanfattning och slutsatser *Lakartidn* 69 1917 1972
10 Klackenberg G A prospective longitudinal study of children Data on psychic health and development up to 8 years of age *Acta Paediatr Scand* Suppl 224 1971
11 Kohler E M *Fyraarsbarn och deras foraldrar Del III Barnets och uppfostrarens beteenden* Report from the Institute of Education (Mimeograph) University of Lund 1971 No 30
12 Köhler L Health control of four year-old children An epidemiological study of child health *Acta Paediatr Scand* Suppl 235 1973

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SERUM LEVELS OF PENICILLIN V AFTER ORAL ADMINISTRATION OF PEDIATRIC PREPARATIONS TO HEALTHY SUBJECTS

H ROLLAG JR T MIDTVEDT and S WETTERHUS

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ABSTRACT Rollag jr H Midtvedt T and Wetterhus S (Kaptein W Wilhelmsen og Frues Bakteriologiske Institutt Rikshospitalet Oslo and the Medical Department Central Hospital of Akershus Lørenskog Norway) Serum levels of penicillin V after oral administration of pediatric preparations to healthy subjects Acta Paediatr Scand 64 421 1975 —The bioavailability of nine commercial pediatric preparations of penicillin V was tested in a double blind cross-over fashion on ten healthy student nurses who were given 1 mill I U of the various preparations The serum concentrations were determined using the paper disc method of AB Biodisc Sweden The preparations could be divided into two different groups (1) the mixtures (2) the effervescent tablets substance for drops and granulate This classification is based upon the mean peak serum levels obtained With one exception the peak serum levels in group 2 were significantly higher than in group 1 2 4 and 6 hours after ingestion there were no differences in the serum levels indicating that none of the preparations gave sustained high serum levels The results presented indicate that the preparations in group 2 should be preferred

KEY WORDS Penicillin V pediatric preparations bioavailability

Penicillin V the most widely used of all antibiotics is marketed in the form of tablets and fluid preparations Given as tablets most preparations are well absorbed and give acceptably high serum concentrations (3 6) However in pediatric praxis there is a need for pleasant tasting mixtures or other kinds of fluid preparations In Norway the mixtures are by far the dominant pediatric preparations of penicillin V (5) Earlier studies have shown that some of these mixtures are poorly absorbed leading to difficulties in maintaining adequate serum levels (4) Therefore the pharmaceutical industry intends to introduce some new formulations of their pediatric preparations on the Norwegian market

The present study was performed in order to compare the bioavailability of new and old preparations of penicillin V for pediatric use that are on sale or intended to be sold in Norway

MATERIALS AND METHODS

Ten healthy student nurses participated in this study The subjects ranged in age from 20–24 years and in weight from 56–74 kg None of the subjects had any history of significant gastrointestinal hepatic or renal disease or of hypersensitivity to penicillins Screening physical examinations and chemical laboratory determinations performed one week before the study revealed no significant abnormalities No other medication was allowed during the investigation period One of the participants had to be excluded from the study after the third day on account of diarrhoea and a general rash A second one left for personal reasons after the fifth day Both were replaced by persons of same age and weight

The experiments were organized in a double blind cross-over fashion The nine pediatric preparations and the tablet of penicillin V that were investigated are listed in Table 1 With the two exceptions mentioned above each person was tested with all ten preparations and each served as her own control

13 Miller F J W Court S D M Walther W S & Knox E G *Growing up in Newcastle upon Tyne* Oxford University Press London 1960
14 Nilsson L *Slutrapport fran försoket med uppsokande verksamhet vid barnavårdscentralerna Handens sjukhus Haninge kommun* (Mimeograph) Socialstyrelsen Stockholm 1973
15 North A F Pediatric care in Project Head Start *In* J Hellmuth (ed) *The disadvantaged child* Brunner & Mazel Seattle 1968 Vol 2 p 93
16 North A F Research issues in child health A Head Start research seminar I Introduction and overview *Pediatrics 45* 690 1970
17 Thomas A Chess S & Birch H *Temperament and behaviour disorders in children* New York University Press New York 1968
18 Vuille J C Den allmanna halsokontrollen av fyraåringar Uppspårande av somatiska avvikelser och psykologiska problem *Lakartidn 68* 456 1971
19 Wallace H M Children and youth projects and related comprehensive health care programs *Clin Pediatr 10* 487 1971
20 *Ekonomiskt stod till barnfamiljer* Betankande utgivet av familjepolitiska kommitten SOU 1972 34
21 *Forskolan* Betankande avgivet av 1968 års barnstugeutredning SOU 1972 27
22 *Halsokontroll av fyraåringar* Socialstyrelsen redovisar 1968 2

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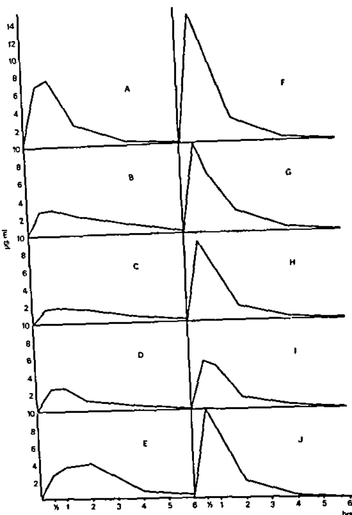


Fig 1 Serum concentrations of penicillin V after an oral dose of 1 mill I U of nine pediatric preparations and a tablet For abbreviations see Table I The curves represent the means of values observed in ten subjects

occurrence of one or more good or bad absorbers of penicillin since the mean peak serum level for each of the 8 persons tested on all the preparations ranged from 5 5 to 8 6 µg/ml with 6 8 as the mean value

DISCUSSION

The present study was undertaken in order to compare the bioavailability of various pediatric preparations of penicillin V The data obtained showed great variations in the bioavailability of the ten preparations The mixtures all showed low peak serum levels occurring one hour or more after ingestion whereas the newer preparations tested (F G H I and J) showed peak serum levels at least twice the levels obtained using mixtures and occurring within one hour after ingestion (Tables 2 and 3)

The importance of high maximum serum concentrations versus prolonged serum concentrations has long been a controversial question (1) It is generally accepted however that an increase in the serum concentration increases the diffusion of penicillin from the blood vessels out in the tissue where the infections are located

The present data show that in all preparations actually investigated comparable serum levels were found 2 hours after ingestion Since comparable levels were also found after 4 and 6 hours no preparations have sustained serum

Table 1 *Code tradename formulation form of penicillin V salt and declared content of peni cillin V of the nine pediatric preparations and the tablet (prep A) used in the study*

Code	Trade name	Formulation	Penicillin salt	Declared content of penicillin V
A	Apocillin	Tablet	Potassium salt	1 000 000 I U ±10%/tabl
B	Novo Fenoxypen	Mixture	Potassium salt	50 000 I U ±10%/ml
C	Calcipen	Mixture	Calcium salt	50 000 I U ±10%/ml
D	Weifapenin	Mixture	Benzantin salt	60 000 I U ±10%/ml
E	Novo Fenoxypen	Mixture (new formulation)	Potassium salt	50 000 I U ±10%/ml
F	Novo Fenoxypen	Granulate	Potassium salt	250 000 I U ±10%/portion
G	Apocillin	Powder for aqueous drops	Potassium salt	1 000 000 I U ±5%/portion
H	Rocilin	Powder for aqueous drops	Potassium salt	3 200 000 I U ±10%/6 5 ml
I	Mekos	Effervescent tabl	Potassium salt	0 24 (0 23–0 25) g/tabl
J	Calicipen	Effervescent tabl	Calcium salt	0 20 g±10%/tabl

* According to the manufacturer

The subjects fasted overnight were given 1 mill I U of the drug together with 100 ml of water and fasted an additional half hour Capillary blood from the finger was collected after 0 ½ 1 2 4 and 6 hours The samples were centrifuged and stored at +4 C until they were tested for penicillin bioactivity later on the same day Ordinarily at least 3 days elapsed between the tests

The penicillin levels were determined by the paper dis method of AB Biodisc Stockholm Sweden utilizin *Micrococcus luteus* ATCC 9341 as test organism an Antibiotic Medium No 1 BPSA (Difco) (?) From eac sample three parallel assays were made and the mea value taken as the result

Table 2 *Serum concentrations (µg/ml) means and ranges after oral administration of nine pediatric preparations and a tablet (prep A) to ten healthy persons*

The dose given was 1 mill I U

	½	1	2	4	6 hours
A	6 8 (1 4–9 5)	7 5 (3 3–11 2)	2 3 (0 8–3 5)	0 5 (0–1 3)	0 (0–0)
B	2 7 (1 3–5 2)	2 8 (1 5–3 9)	2 1 (0 9–3 8)	1 0 (0 3–1 4)	0 2 (0–1 0)
C	1 6 (0 4–3 2)	1 7 (0 7–2 5)	1 5 (0 6–2 6)	0 7 (0 2–1 4)	0 2 (0–1 6)
D	2 4 (0 8–4 4)	2 6 (1 5–4 8)	1 0 (0 5–1 5)	0 4 (0–1 1)	0 1 (0–0 5)
E	2 6 (1 0–4 8)	3 4 (1 8–7 5)	3 9 (1 5–7 8)	0 7 (0–1 3)	0 3 (0–1 2)
F	14 6 (3 5–24 5)	10 4 (4 8–17 0)	2 7 (1 0–7 8)	0 3 (0–0 7)	0 1 (0–0 8)
G	10 0 (5 8–14 0)	6 5 (3 1–9 3)	2 4 (0 6–7 2)	0 3 (0 2–0 7)	0 (0–0 2)
H	9 0 (0 6–16 0)	6 3 (4 8–9 0)	1 7 (1 1–2 6)	0 3 (0–0 8)	0 (0–0)
I	5 5 (1 6–10 7)	5 0 (1 9–14 8)	1 2 (0 4–1 9)	0 3 (0–0 9)	0 (0–0)
J	10 0 (5 1–18 0)	7 3 (2 7–14 0)	1 8 (0 6–3 7)	0 2 (0–0 4)	0 (0–0)

RESULTS

The serum concentrations of penicillin bio activity (means and range) of the 10 prepara tions investigated are presented in Table 2 Th means are visualized in Fig 1 The means an the standard deviations of the peak serum levels regardless of the time of occurrence ar presented in Table 3

As may be seen from the data recorded th preparations can be divided in two groups (1 the mixtures (2) the others The prepara tions in group 1 reached their maximum serum levels an hour or two after ingestion wherea the preparations in group 2 reached their max imum serum levels within one hour Further al preparations in group 2 except prep I reache significantly (Student test) higher peak serum levels than the preparations in group 1 (Tabl 3) Prep I had a somewhat but not significantl higher peak serum level than the preparation in group 1 (Table 3)

In each preparation the serum levels wer found to vary within a wide range (Table 2) However these variations were not due tc

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ASYMPTOMATIC BACTERIURIA IN SCHOOL GIRLS

I Clinical and Laboratory Findings

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ABSTRACT Lindberg U Claesson I Hanson L Å and Jodal U (Departments of Paediatrics and Roentgenology the Children s Hospital and the Department of Immunology Institute of Medical Microbiology University of Goteborg Goteborg Sweden) Asymptomatic bacteriuria in schoolgirls I Clinical and laboratory findings Acta Paediatr Scand 64 425 1975 —Among 116 schoolgirls with asymptomatic bacteriuria detected at urinary screening renal parenchymal reduction was found in 10.3% while reflux was found in 20 7% Only 30% of the 116 patients had a history referable to earlier urinary tract infection and there were remarkably few girls with an increased sedimentation rate (4 4%) C reactive protein (9 5%) pyuria (25 8%) or lowered concentrating capacity (3 4%) at the time of detection of their bacteriuria No method was found efficient in predicting lesions on the pyelogram and urethrocystogram but determination of renal concentrating capacity and C reactive protein was of some value in predicting parenchymal reduction The girls with pyelonephritic changes on the pyelogram had a mean renal concentrating capacity significantly lower than the girls without changes The concentrating capacity of the girls with reflux but without renal scarring and those bacteriuric patients without radiologically demonstrated defects did not differ significantly from the age related normal values

KEY WORDS Asymptomatic bacteriuria schoolgirls clinical roentgenological findings renal concentrating capacity pyuria C reactive protein sedimentation rate

Urinary tract infection (UTI) detected by screening is usually referred to as asymptomatic bacteriuria (ABU) The patients have either no symptoms or such minor symptoms that a doctor has not been consulted The prevalence in a population of healthy schoolchildren is 1 1% in girls and 0 03% in boys according to Kunin et al (8 9) Savage et al found a prevalence of 1 6% in 5 year old girls screened on entry to primary school (16) In a study from Lund 0 8% of 4-year-old girls and 1 5% of schoolgirls had ABU (11 17) In Goteborg a prevalence of 0 7% was found among 4300 schoolgirls (5) Since the long term consequences of ABU in childhood are uncertain its association with renal infection vague and the development of renal parenchymal reduction as well as the need for and/or best form of treatment is still unknown we wanted to pursue a detailed investigation of the clinical characteristics and course the value of various diagnostic measures and the therapy of patients with ABU This first paper is concerned with the roentgenological findings and their relation to pyuria sedimentation rate C reactive protein (CRP) concentrating capacity and a history of earlier infections

MATERIAL AND METHODS

Since 1970 about 19000 girls a year are routinely screened for bacteriuria in Göteborg schools when 7 11 14 and

Table 3 *Means and standard deviations of peak serum levels (μg/ml) regardless of the time of occurrence after oral administration of penicillin V as nine pediatric preparations and a tablet (prep A)*

The dose given was 1 mill I U

A	9 0±1 5	F	16 4±4 0
B	3 4±0 9	G	10 3±2 6
C	2 0±0 6	H	10 0±3 6
D	3 0±0 9	I	6 9±2 3
E	4 2±1 8	J	11 7±3 9

concentration levels The preparations in group 2 were generally found to give higher and more rapidly rising serum levels than the preparations in group 1 In fact the serum levels obtained with the preparations in group 2 were of the same order of magnitude as the levels obtained using a tablet preparation (prep A)

In all preparations in group 2 the peak serum levels were at least twice (up to 6 times) the levels obtained by the best mixtures tested

From a pharmacological point of view the present investigation indicates that pediatric preparations of penicillin V should be chosen from among those in group 2 However further investigations are needed in order to elucidate their clinical usefulness

REFERENCES

1 Chisholm G D Waterworth P M Calman J S & Garrod L P Concentration of antibacterial agents in interstitial tissue fluid *Br Med J 1* 569 1973
2 Jalling B Malmborg A S Lindman A & Boreus L O Evaluation of a micromethod for determination of antibiotic concentrations in plasma *Europ J Cl Pharmacol 4* 150 1972
3 Johnsgård M & Torud Y Absorpsjon av fenoxymetylpenicillin fra Femepentabletter *Norges Apotekerforenings Tidsskrift* 79 464 1971
4 Lund J S Sardemann H & Christensen H B Absorptionen af penicillin efter indgift av V-penicillin mixturer *Ugeskr Læger 127* 1761 1965
5 *Terapeutisk kjemisk legemiddelregister 1974* Norsk Medisinaldepot
6 Walter A M & Heilmeyer L *Antibiotika Fibel* G Thieme Verlag Stuttgart 1969 2nd ed p 170

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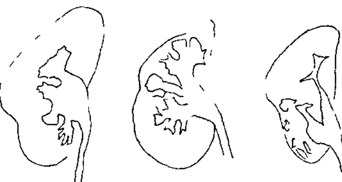


Fig 1 Classification of parenchymal reduction + a minor shift of the renal outline ++ a distinct parenchymal reduction +++ an almost complete reduction of parenchyma corresponding to a deformed calyx group From patients 6 9 and 11 in Table 1

(p=0 51 for febrile or afebrile infection and 0 22 for febrile infection) (Table 3) However the p value for a past history of *febrile* UTI and abnormal findings on IVP *or* MCU was 0 06

A history of primary enuresis was found in 22 girls (18 9% 21 nocturnal and one diurnal) 9 of whom (7 7%) still had a nocturnal enuresis when admitted Of these 9 girls one was found in the group with parenchymal reduction and 2 among the girls with reflux but without kidney changes (Table 3) Secondary enuresis was present in 9 girls (7 7% 2 nocturnal and 7 diurnal) all without renal parenchymal reduction or reflux (Table 3) Neither on IVP nor on MCU were there significantly more abnormal findings ($p \geq 0$ 27) in girls with enuresis (Table 3)

Physical examination

All children appeared well and general examination did not reveal any abnormalities None had an elevated blood pressure On the Karlberg et al growth chart for Swedish girls 6 to 18 years old (7) all of the patients were normally distributed between ±2 S D for height and weight except for one girl who was between −2 and −3 S D for weight

Laboratory findings

E coli were found in the urine of all except one girl in whose urine *Klebsiella Aerobacter* was isolated Pyuria i e white cell excretion >50 cells/mm³ was found in 30 girls (25 8%) Six of the 12 patients with renal scarring and 4 of the 13 girls with reflux but without detectable renal

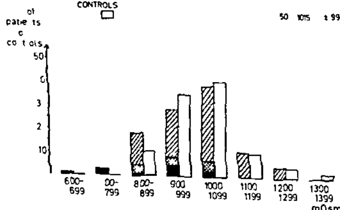


Fig 2 The renal concentrating capacity in 116 girls with ABU and 50 girls without bacteriuria.

16 years old using a chemical test the BM Test Nitnte (Boehringer Mannheim GmbH Mannheim BRD) During the autumn of 1971 and throughout 1972 schoolgirls 7–15 years old were referred to the Paediatric Department at the Children s Hospital for further examination because of at least two positive tests in the screening program Of these 116 with significant bacteriuria (≥10^5/ml) with the same organism in two consecutive urine samples and no symptoms at the first visit to the hospital were included in the study In addition to the controls for renal concentrating capacity presented by Winberg (19) a group of 50 girls of the same ages were for comparison chosen from patients with earlier UTI They had had negative urine cultures during the preceding 6 months Intravenous pyelography and micturating cysto-urethrography performed in 39 of these 50 girls were normal

A careful history was obtained to reveal symptoms of previous UTI and enuresis (involuntary urination after 5 years of age) A physical examination including measurement of height weight and blood pressure was done

Urine was collected as a midstream sample after careful cleaning of the external genitalia and was kept at +4 C until semi quantitatively cultured (12)

To obtain a second sample the patients were instructured to collect a morning midstream specimen which was cultured with a dipslide (Uricult® Orion Pharmaceutical Co Helsinki Finland) at home The slide was sent by mail to the laboratory and read after incubation overnight in 37 C Classification of the bacteria and 0-grouping of *E coli* were performed as earlier described (12)

The urinary white cell excretion was determined by counting the leucocytes in uncentrifuged urine using a Fuchs Rosenthal chamber A count of >50 cells/mm³ was regarded as pathologic (2) Blood samples were obtained and analysed for micro-sedimentation rate and CRP (14) A micro-sedimentation rate >20 mm/h and CRP >10 µg/ml were considered abnormal (6)

The concentrating capacity of the kidneys was estimated by freeze point reduction in two consecutive urine samples obtained at home after fluid deprivation for at least 15 hours The highest value was noted The age related normal values of Winberg (19) were used as a reference If the concentrating capacity was <814 mOsm/l the test was repeated

Intravenous pyelography (IVP) and micturating cysto urethrography (MCU) were performed on all 116 girls with ABU The IVP was appraised with regard to pyelonephritic changes using the criteria of Hodson (4) Patients with calyceal deformation or dilatation were grouped according to the number of calyceal groups involved The degree of parenchymal reduction was estimated according to a scale in which + meant a minor shift of the renal outline ++ a distinct parenchymal reduction and +++ an almost complete reduction of parenchyma corresponding to a deformed calyx group (Fig 1) Maximum length of the kidneys was measured on roentgenograms obtained without compression and with the patient in a supine position

In order to reduce the radiation dose to the gonads during the MCU and to attain diagnostic information during the filling as well as micturition fluoroscopy and 70 mm ciné technique were used As a rule exposures in both antero and lateral projection were obtained Exposures were always made before micturition in order to reveal reflux occurring during filling of the bladder The reflux was rated in four degrees I reflux in a non-dilated ureter and not reaching the kidney pelvis II into the pelvis but without dilatation III into the kidney pelvis with dilatation of the pelvis and ureter and IV into the kidney pelvis with extreme dilatation of the pelvis and ureter (21)

Statistical methods

The Wilcoxon test for two samples and Fisher s exact test for 2×2 contingency tables were employed

RESULTS

Radiological investigations

IVP revealed 12 children (10 3%) with calyceal changes and parenchymal reduction suggestive of previous pyelonephritis (Fig 1 and Table 1) In 2 girls there were bilateral changes Reflux was diagnosed in 11 of these 12 girls (Tables 1 and 2) Another 13 girls with normal IVPs had reflux (11 2%) making a total of 24 girls with this finding (20 7%) The reflux was bilateral in 10 girls The mean degree of reflux did not differ between the groups with and without renal parenchymal changes (Table 2) Five girls had a double collecting system (4 3%) and a diverticulum of the urinary bladder was found in 3 girls (2 6%)

History

Of the 12 girls with parenchymal reduction of the kidney 2 (17%) had previously been treated for febrile UTI and 3 for afebrile infections (Table 3) Among the 13 patients with reflux without detectable changes of the kidneys 2 (15%) had had febrile and 2 afebrile UTI (Table 3) In the group without parenchymal reduction or reflux (91 girls) 4 (4%) had been treated for febrile UTI and 22 girls had had symptoms of afebrile infections Thus 30 1% of the group had a history that could be related to UTI Abnormal findings on IVP were not significantly more common in children with a past history of UTI (p =0 34 for febrile or afebrile infection and 0 19 for febrile infection) Neither were MCU abnormalities significantly more common in this group

Laboratory findings			
/BC/mm ·ine	Sedimentation rate (mm/hour)	C reactive protein (μg/ml)	Concentrating capacity (mOsm/l)
30	13	0	947
44	9	0	638
77	17	0	907
11	7	0	869
16	16	0	1 041
05	13	0	1 000
70	9	0	764
80	9	39	974
10	40	70	969
9	7	11	706
17	8	0	901
70	30	37	735

changes had a mean of 965±97 mOsm/l a value not statistically different ($p>0.05$) from patients without roentgenological changes or that of the 50 girls without bacteriuria who had

Table 3 *Past history of febrile and afebrile UTI and primary and secondary enuresis on admission correlated to parenchymal reduction (11/12 with reflux) and reflux without parenchymal reduction in 116 schoolgirls with ABU*

History	Number of patients	Radiology: Parenchymal reduction	Radiology: Reflux only
Earlier febrile or afebrile UTI			
Yes	35	5	4
No	81	7	9
		$p=0.34$	$p=0.51$
Earlier febrile UTI			
Yes	8	2	2
No	108	10	11
		$p=0.19$	$p=0.72$
Primary enuresis on admission			
Yes	9	1	2
No	107	11	11
		$p=0.63$	$p=0.27$
Secondary enuresis on admission			
Yes	9	0	0
No	107	12	13
		$p=1.00$	$p=1.00$

Table 4 *Pyuria increased sedimentation rate and C reactive protein correlated to parenchymal reduction (11/12 with reflux) and reflux without parenchymal reduction in 116 schoolgirls with ABU*

Laboratory findings	Number of patients	Radiology: Parenchymal reduction	Radiology: Reflux only
Pyuria			
>50 WBC/mm	30	6	4
<50 WBC/mm	86	6	9
		$p=0.05$	$p=0.53$
Sedimentation rate			
>20 mm/hour	5	2	1
< 0 mm/hour	111	10	12
		$p=0.07$	$p=0.44$
C reactive protein			
>10 μg/ml	11	4	1
<10 μg/ml	105	8	12
		$p=0.01$	$p=0.75$

1015±99 mOsm/l (Fig 2) Four patients all with pyelonephritic changes on the IVP had less than 814 mOsm/l (Table 1)

In only one girl were all laboratory parameters abnormal i e leucocytes 70/mm³ urine micro-sedimentation rate 30 mm/h CRP 37 μg/ml and concentrating capacity 735 mOsm/l She had roentgenological changes indicating previous pyelonephritis (No 12 Table 1)

DISCUSSION

The frequency of roentgenological changes (10 3% parenchymal reduction and 20 7% reflux) and past history of urinary tract infection found in this study are in good agreement with those of Kunin et al in their long term study of the epidemiology and natural history of UTI in schoolchildren in Virginia USA (10) However Savage et al have recently reported a higher rate of roentgenological changes on covert (=asymptomatic) bacteriuria in 5 year-old girls than noted in the present study (16) They found radiological evidence of pyelonephritis in 23% while 35% had reflux Our lower incidence of renal scarring and reflux

Table 1 *Radiological and laboratory findings in 12 girls with ABU and renal parenchymal reduction*

		Intravenous pyelogram						
Patient no	Age	Deformation in one calyx group	Deformation in more than one calyx group	Reduction of parenchyma		Renal length (cm)		Micturition cysto-urethrogram (Degree of r
				Degree	Side	Right	Left	
1	15		+	+++	Bilat	11 5	12	Right II left
2	15		+	+++	Right	13 5	12 5	No reflux
3	15	+		++	Left	12	9	Bilat II
4	14		+	+++	Left	12 5	8	Left II
5	14		+	+++	Left	13 5	6 5	Left I
6	14	+		+	Right	9	11 5	Right I
7	12	+		+	Left	12 5	13	Bilat II
8	11		+	+++	Left	11	10	Left II
9	10		+	++	Right	9	13	Bilat II
10	10		+	++	Bilat	8	10	Bilat II
11	8		+	+++	Right	6	12 5	Right I
12	8		+	+	Right	8 5	10	Right I

* Duplication of pelvis

changes had pyuria (Table 4) Pyuria was thus significantly more common ($p=0\ 05$) in girls with parenchymal reduction In contrast abnormal findings on MCU in patients without renal scarring were not significantly more prevalent ($p=0\ 53$) in children with than without pyuria (Table 4)

Micro sedimentation rate >20 mm was found in 5 girls (4 3%) 2 in the group with kidney damage one in the group with reflux and 2 among the girls without roentgenological changes (Table 4) The correlation between increased micro sedimentation rate and radiological abnormalities was not statistically significant ($p \geq 0\ 07$) (Table 4)

Increased quantity of serum CRP >10 μg/ml was found in 11 girls (9 5%) 4 in the group with renal scarring one in the group wi[th] reflux only and 6 among the girls witho[ut] roentgenological changes (Table 4) Abnorm[al] findings on IVP were significantly more com[mon] ($p=0\ 01$) in children with increased CR[P] but MCU abnormalities were not ($p=0\ 7$[…]) (Table 4)

Renal concentrating capacity

The 116 bacteriuric girls had a mean urinary osmolality of 991±117 mOsm/l after fluid deprivation The 91 girls without renal scarring or reflux had a mean of 1011±89 mOsm/l while the 12 girls with pyelonephritic changes on the pyelogram had 869±125 mOsm/l (Fig 2) a significantly lower value ($p<0\ 01$) The patients with reflux but with no detectable renal

Table 2 *The relation between reflux and renal parenchymal reduction in 116 ABU girls*

Group of patients	No of patients	With reflux	Degree		Side			During filling	During micturition
			I	II	Bilat	Right	Left		
With renal parenchymal reduction	12	11	4	7	5	3	3	8	3
Without renal parenchymal reduction	104	13	5	8	5	5	3	7	6
Total	116	24	9	15	10	8	6	15	9

...aboratory findings			
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...1	7	0	869
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might be explained by the fact that since 1959 a prospective study on the prognosis of symptomatic urinary tract infection has been conducted at the Children s Hospital Goteborg (1) Relapsing infections in patients included in this program are treated with antibiotics and therefore seldom appear as bacteriuric in the school screening

Analysis of the renal concentrating capacity showed that the girls with renal parenchymal reduction had a mean concentrating capacity significantly lower than patients without infections during the last 6 months and ABU patients without any roentgenological changes A trend to lower values was found in the girls with reflux but without parenchymal reduction Savage et al also found a lowered concentrating capacity in 5 year old children with ABU when there was radiological evidence of parenchymal disease (15) and similar results were obtained by Clark et al in women when renal infection could be demonstrated by ureteric catheterization (3)

Methods to predict the presence of roentgenological changes in the ABU patients with the intention to reduce the number of roentgenological examinations would be of value The laboratory tests used here seemed poor in this respect Determination of renal concentrating capacity and CRP were the best methods The four girls with renal concentrating capacity <814 mOsm/l all had parenchymal reduction on IVP while 4 of 11 girls with increased CRP had these changes None of the tests employed could predict lesions on the MCU in girls without renal parenchymal reduction

The roentgenological findings in the present study suggest that as many as 10% of the girls with ABU have had infections causing renal parenchymal reduction However a history of febrile or afebrile UTI was obtained in only 5 of the 12 girls with parenchymal reduction But since symptoms of UTI in early childhood are often uncharacteristic and restricted to fever UTI causing renal damage may have been overlooked In non pregnant women with ABU more than 90% had experienced symptoms of UTI and 20% of the patients had had febrile UTIs in the past (18) while in the present study a history of UTI symptoms was found in only 30% of the girls and a history of febrile infections in 7%

Few girls with an increased sedimentation rate pyuria CRP or reduced concentrating capacity were found Even in the patients with parenchymal reduction 4 out of 12 had normal findings in this respect and in the patients with reflux but without parenchymal reduction the tests were normal in 8 out of 13 A change in the pyelogram does not necessarily imply an active pyelonephritis when bacteria are present in the urine But in 4 cases the renal damage was combined with a low concentrating capacity which became normal after clearing of the bacteriuria by antibiotic treatment (13) This indicates that among the ABU children some may have ongoing injuries requiring detection and treatment Furthermore the data suggest that significant bacteriuria diagnosed in school age does not detect urinary tract infection at an early stage of its natural history Whether screening early in childhood can prevent renal damage is unknown Even in a group of children followed from the first attack of pyelonephritis and treated at each noticed recurrence renal scarring developed in 7% (20)

Continued follow up of the present series of bacteriurics treated and untreated may provide further information on the natural history of ABU and the need for a screening program in school age

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REFERENCES

1 Bergstrom T Lincoln K Redin B & Winberg J Studies of urinary tract infections in infancy and childhood X Short or long term treatment in girls with first and second time urinary tract infections uncompli

cated by obstructive urological abnormalities Acta Paediatr Scand 57 186 1968
2 Braude H Forfar J O Gould J C & McLeod J W Cell and bacterial counts in the urine of normal infants and children Br Med J IV 697 1967
3 Clark H Ronald A R Cutler R E & Turck M The correlation between site of infection and maximal concentrating ability in bacteriuria J Inf Dis 120 47 1969
4 Hodson C J & Wilson S Natural history of chronic pyelonephritis scarring Br Med J II 191 1965
5 Jodal U Lincoln K & Lindberg U To be published
6 — Level diagnosis of symptomatic urinary tract infections in childhood Acta Paediatr Scand 64 201 1975
7 Karlberg P Engstrom I Klackenberg G Klackenberg Larsson I Lichtenstein H Svennberg I & Taranger J Growth charts for height weight and head circumference Lakartidningen 35 2960 1973
8 Kunin C M Southall I & Paquin A Jr Epidemiology of urinary tract infections A pilot study of 3 057 school children N Engl J Med 263 817 1960
9 Kunin C M Zacha E & Paquin A Jr Urinary tract infections in children I Prevalence of bacteriuria and associated urologic findings N Engl J Med 266 1 87 196
10 Kunin C M Deutscher R & Paquin A Jr Urinary tract infections in school children An epidemiologic clinical and laboratory study Medicine 43 91 1964
11 Kohler L Fritz H & Scherstén B Health control of four year-old children A study of bacteriuria Acta Paediatr Scand 61 289 1972
12 Lincoln K Lidin Jansson G & Winberg J Resistant urinary infections resulting from changes in resistance pattern of faecal flora induced by sulphonamide and hospital environment Br Med J III 305 1970
13 Lindberg U Asymptomatic bacteriuria in school girls V The clinical course and response to treatment To be published
14 Nilsson L Å Comparative testing of precipitation method for quantitation of C reactive protein in blood serum Acta Pat Microbiol Scand 73 129 1968
15 Savage D C L Wilson M I & Fee W M Renal concentrating ability in 5-year-old girls with covert bacteriuria Arch Dis Child 47 141 1972
16 Savage D C L Wilson M I McHardy M Dewar D A E & Fee W M Covert bacteriuria of childhood A clinical and epidemiological study Arch Dis Child 48 8 1973
17 Scherstén B Fritz H & Kohler L Subnormal concentration of urinary glucose as a sign of urinary tract infection in children Acta Paediatr Scand 54 Suppl 177 54 1967
18 Sussman M Asscher A W Waters W E Evans Joy A S Campbell H Evans K T & Williams J E Asymptomatic bacteriuria in non pregnant woman I Description of a population Br Med J I 799 1969
19 Winberg J Determination of renal concentrating capacity in infants and children without renal disease Acta Paediatr Scand 48 318 1959
20 Winberg J Bergstrom T & Hanson L Å Neue Gesichtspunkte zur Harnwegsinfektion mit E coli Monatsschr Kinderh 119 506 1971
21 Winberg J Larsson H & Bergström T Comparison of the natural history of urinary infection in children with and without vesico-ureteric reflux In P Kincaid Smith and K F Fairley (eds) Renal infection and renal scarring Mercedes Melbourne 1971 p 293

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ASYMPTOMATIC BACTERIURIA IN SCHOOLGIRLS

II Differences in Escherichia Coli Causing Asymptomatic and Symptomatic Bacteriuria

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ABSTRACT Lindberg U Hanson L Å Jodal U Lidin Janson G Lincoln K and Olling S (Department of Paediatrics and Departments of Immunology and Clinical Bacteriology Institute of Medical Microbiology University of Goteborg Goteborg Sweden) Asymptomatic bacteriuria in schoolgirls II Differences in *Escherichia coli* causing asymptomatic and symptomatic bacteriuria Acta Paediatr Scand 64 432 1975 —Three hundred and forty three *E coli* strains isolated from the urine of patients with asymptomatic bacteriuria (ABU) symptomatic cystitis or pyelonephritis were analysed with regard to O group distribution and sensitivity to the bactericidal effect of normal serum Strains of O groups 1 2 4 6 7 16 18 and 75 were found in 31.3% in ABU in 58 7% in cystitis and in 79 8% in pyelonephritis Spontaneous agglutination was noted in 45 2% of ABU 6 5% of cystitis and 1 7% of pyelonephritis strains The strains from patients with ABU were significantly more sensitive to the bactericidal effect of normal serum than were those from patients with symptomatic urinary tract infection In some patients with untreated ABU changes in the characteristics of the urinary strains isolated were noted The strains tended to become spontaneously agglutinating as well as more sensitive to the bactericidal activity of normal serum The strains found in patients with ABU probably had an altered cell wall compared with those found in patients with symptomatic infections such that they produce fewer symptoms and possibly be less virulent

KEY WORDS *E coli* O groups bactericidal effect of normal serum asymptomatic bacteriuria symptomatic cystitis and pyelonephritis girls

Urinary tract infection (UTI) is common in man, even in the absence of any detectable malformation of the urinary tract (20) Recurrences are common and renal parenchymal lesions appear in some patients (1 10) The severity of symptoms varies and in many patients there are no symptoms indicating UTI (5, 9 17) Why some patients lack symptoms is not known but a development of endotoxin tolerance (12) and a change in the infecting bacteria (13) have been suggested as possible factors of importance

As part of a longitudinal study of the host–parasite relationship in UTI this investigation characterizes the infecting bacteria isolated from schoolgirls with asymptomatic bacteriuria (ABU) with respect to O group and resistance to the bactericidal activity of normal serum For comparison *E coli* strains from girls with their first known symptomatic UTI are similarly analysed

MATERIAL AND METHODS

The patients were 116 schoolgirls 7–15 years old with ABU detected in a school screening program during 1971 and

Table 1 *Distribution of E coli O antigen groups*

Group	Pyelo-nephritis N		Cystitis N	%	ABU N	%
O1	7	5 9	1	0 9	10	8 7
O	19	15 9	10	9 2	5	4 3
O4	?3	19 3	7	6 4	1	0 9
O6	11	9 3	16	14 7	?	1 7
O7	11	9 3	1?	11 0	?	1 7
O16	13	10 9	0	0 0	6	5 2
O18	8	6 9	5	4 6	5	4 3
O 5	3	? 5	13	11 9	5	4 3
Subtotal	95	79 8	64	58 7	36	31 3
O8	1	0 8	6	5 5	0	0 0
O14	0	0 0	0	0 0	4	3 6
O25	1	0 8	9	8 3	3	6
O83	1	0 8	4	3 7	1	0 9
Subtotal	3	2 5	19	17 5	8	7 0
Other groups	10	8 4	13	11 °	7	6 1
Not agglutinable (ON)	9	7 6	6	5 5	12	10 4
Spontaneously agglutinating (SA)	2	1 7	7	6 5	5?	45 ?
Total	119		109		115	

One or two each of the following O3 O5 O10 O13 O15 O17 O20 O21 O23 O55 O77 O78 O8? O85 O99 O11? O117 O147

* Eleven ABU strains and one cystitis strain also reacted against O 14 antiserum

1972 (9) Each patient had two positive nitrite tests verified by two urine samples with ≥100 000 bacteria/ml and the same type of bacteria in both cultures The radiology and estimation of renal concentrating capacity erythrocyte sedimentation rate (ESR) C reactive protein (CRP) and leu ocytes in urine have been reported earlier (9) Twelve girls were found to have renal parenchymal reduction

Strains for comparison were selected from our collection of *E coli* strains They had all been isolated from girls with their first known UTI seeking medical attention because of acute symptoms and treated by one of us (U J) during the years 1968 to 197? One hundred and nine girls aged 3 to 15 years had cystitis as judged by the criteria of acute frequency and burning temperature not exceeding 38 0°C a bacterial count of ≥100 000 bacteria/ml of urine and normal renal concentration capacity in relation to age (19) One hundred and nineteen girls 3 months to 15 years old had pyelonephritis as judged by the criteria fever >38 0°C a bacterial count of ≥100 000 bacteria/ml of urine and one or both of ESR>?0 mm/h and decreased concentrating capacity in relation to age (19)

From each girl the strain at discovery of bacteriuria was analysed. Twenty-three of the 116 girls with ABU were not treated for their bacteriuria From these isolates from consecutive cultures 3 to 6 months apart were also analysed

O grouping

A simplified *E coli* O grouping was performed as earlier described (7) The antisera were as follows In a first stage 10 monovalent sera to the O antigen groups 1 ? 4 6 7 8 16 18 25 and 75 were used If a distinct agglutination did not occur monovalent antiserum to O14 was tried in addition to two sets of polyvalent antisera to 56 less common O groups Cultures that were nonspecifically agglutinated by all ten sera in the first stage were characterized as spontaneously agglutinating and designated SA Such strains frequently formed rough colonies on the Drigalski agar plate Strains that were not agglutinated by the available sera and did not agglutinate spontaneously were characterized not agglutinable and designated ON

The bactericidal test has been reported earlier (13) The bacterial suspension to be tested was mixed with normal human serum and incubated at 37°C for 30 minutes Brain Heart Infusion broth was then added to inhibit further bactericidal activity and to obtain optimal growth conditions and the suspension was incubated at 37°C for 3–5 hours The percentage of surviving bacteria was determined by densitometric comparison with three standard tubes without serum in which the test organisms had been diluted to 50% 10% and 1% of the suspension used in the test The results of the analyses were rated according to the following scale 0 indicates <50% of the bacteria killed 1 indicates 51–90% killed 2 indicates 91–99% killed and 3 indicates >99% killed

For *statistical evaluation* the chi square test was employed

RESULTS

E coli O groups

All bacteria isolated were *E coli* except one *Klebsiella* strain from an ABU patient The distribution of *E coli* O antigen groups found in the 343 *E coli* is presented in Table 1 Strains of O groups 1 2 4 6 7 16 18 and 75 were found in 31 3% of ABU 58 7% of cystitis and in 79 8% of pyelonephritis isolates These figures differed significantly from each other ($p<0\ 001$) Spontaneously agglutinating (SA) strains were isolated from 45 2% of girls with ABU and were significantly more common ($p<0\ 001$) than among girls with symptomatic cystitis (6 5%) or pyelonephritis (1 7%) *E coli* O 14 was found only in the ABU group In addition a reaction with O 14 antiserum was found in eleven of the 52 SA strains in the ABU group and in one of the seven SA strains in the cystitis group

Table 2 *Sensitivity to the bactericidal effect of normal human serum*

	Pyelonephritis						Cystitis						ABU					
	Number of strains						Number of strains						Number of strains					
	Rating					Mean	Rating					Mean	Rating					Mean
	0	1	2	3	Total	rating	0	1	2	3	Total	rating	0	1	2	3	Total	rating
Group O1 O2 O4 O6 O7 O16 O18 O75	64	18	9	4	95	0.5	52	7	2	3	64	0.3	9	3	9	15	36	1.8
Other groups	17	3	1	1	22	0.4	24	7	4	3	38	0.6	4	–	1	21	26	2.5
Spontaneously agglutinating	1	1	–	–	2	0.5	2	–	1	4	7	1.5	1	2	4	44	51	2.6
Total	82	22	10	5	119	0.5	78	14	7	10	109	0.5	14	5	14	80	113	2.4

Two strains not possible to test because of too slow growth

Sensitivity to the bactericidal effect of normal human serum

The number of *E coli* strains sensitive (rating value 3) to the bactericidal effect was significantly ($p<0.001$) larger in patients with ABU (80/113 70.3%) than in girls with cystitis (10/109 9.2%) or pyelonephritis (5/119 4.2%) (Table 2) Two strains in the ABU group grew too slowly to be tested

Among the ABU strains those belonging to common O groups were less sensitive (mean rating value 1.8) than the SA strains (rating 2.6) Strains of common O groups from patients with symptomatic cystitis or pyelonephritis were however even less sensitive (rating 0.5 and 0.3 respectively)

Changes in O group typability and sensitivity to bactericidal effect of normal serum in patients followed with untreated ABU

Seventeen of the 23 ABU girls left untreated had strains initially sensitive to the bactericidal effect of normal serum No change to a lower sensitivity was observed during one year s observation In 2 of these patients a change in O group typability to spontaneous agglutination was observed (Fig 1) Six of the 23 untreated ABU girls initially harboured strains of low serum sensitivity rating value 0–1 A change to a higher sensitivity was found in 4 of these 6 strains during follow up (Fig 2) They were however unchanged in O group typability

The strains in 12 girls with ABU and parenchymal reduction

In Table 3 O group and serum sensitivity of the strains from the 12 girls with ABU and parenchymal reduction are presented together with the patients ESR CRP and renal concentrating capacity The frequency of SA strains 7/12 was not significantly different from that among the rest of the ABU strains 45/104 SA strains were found in the 4 patients with transiently decreased renal concentrating

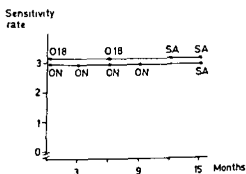


Fig 1 The change in O group typability during follow-up of the bacteria in 2 patients with untreated ABU ON=not groupable with available antisera SA=spontaneously agglutinating

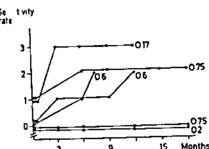


Fig 2 Changes in the sensitivity of the bactericidal effect of normal serum during follow up in 4 of 6 girls with un treated ABU

capacity (<814 mOsm/l) One serum sensitive SA strain occurred in a girl (No 12) with decreased renal concentrating capacity as well as increased CRP and ESR After treatment these signs of parenchymal involvement were absent in all patients (8)

DISCUSSION

This work confirms the findings in an earlier paper (13) that the sensitivity to the bactericidal activity of normal serum differs among *E coli* strains causing different clinical forms of UTI There was also a difference in the distribution of O groups In contrast to the strains causing first time symptomatic infections the ABU strains were more often serum sensitive and spontaneously agglutinating Both these properties probably reflect changes in the bacterial envelope

Lipopolysaccharides are characteristic com ponents of the cell walls of Gram negative bacteria and are probably responsible for most of the symptoms of the host (14) The polysaccharide portion contains the determi nants of the O antigen which provides one basis for the serological classification of the *Enterobacteriaceae* (4) Loss of carbohydrate components by mutation can change the O antigen characteristics of the bacterium result ing in spontaneous agglutination and some times in a rough colony on agar plates The changed cell wall makes the bacterium less virulent although virulence is polygenic in character (16)

The factors involved in the complement dependent bactericidal effect of serum are not well defined It is known that bacterial en dotoxin is able to initiate the alternative path way of complement activation probably via properdin (6 15 18) Perhaps variations in the bacterial envelope make the strains more or less susceptible to the action on complement thus explaining the findings of Mackie & Fink elstein (11) that resistance or sensitivity is a characteristic of the individual strain and not of species

The successively increased sensitivity to bactericidal activity of some strains during follow-up as well as the change from specific to spontaneous agglutination of two strains are in accord with the findings of Bettelheim & Taylor (2) They described degrees of loss variation affecting the somatic O the surface K and the flagellar H antigen of *E coli* strains isolated repeatedly during the course of chronic urinary tract infection in adult women

Factors that may select serum sensitive and spontaneously agglutinating strains in patients with UTIs have not been defined Secretory IgA as well as IgG antibodies against O and K antigens of the infecting bacteria have recently

Table 3 *Properties of strains and clinical laboratory findings in 12 girls with ABU and renal parenchymal reduction*

	E coli strain				
Pat no	O group	Serum sensi tivity	ESR (mm/h)	CRP (μg/ml)	Concentrating capacity (mOsm/l)
1	O 15	0	13	0	947
2	SA		9	0	638
3	ON	3	12	0	907
4	SA	2	7	0	869
5	O 75	3	16	0	1 041
6	SA	3	13	0	1 000
7	SA	3	9	0	764
8	O 2	0	9	39	974
9	O 1	3	40	?0	969
10	SA	1	7	11	706
11	SA	3	8	0	901
12	SA	3	30	37	735

Not possible to test because of too slow growth
ON = not groupable with available antisera
SA = spontaneously agglutinating

been found in the urine of girls with urinary tract infection however (3) and they may be of importance in this respect The strains with detectable O specific side chains may be more affected by such antibodies than the spontaneously agglutinating ones These bacteria may have been selected in ABU patients

The changed strains found in ABU seem to give fewer symptoms than the bacteria with a more complete O antigen found in symptomatic infections However it is unknown if they are less efficient in causing the renal parenchymal reduction that seems to appear in some children with UTI (10, 20) The SA strains may apparently cause a reversible decrease in renal concentrating capacity, as found in 4 of the girls with renal parenchymal reduction But the relation between the permanent kidney damage and the infection detected as a silent pyelonephritis is impossible to assess

Further studies are necessary to evaluate whether the ABU strains are harmless to the host and if left in the urinary tract perhaps protect against more virulent strains (8) Such information is important for the planning of a screening program as well as treatment and follow up

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REFERENCES

1 Bergstrom T Larson H Lincoln K & Winberg J Studies of urinary tract infections in infancy and childhood XII Eighty consecutive patients with neonatal infection *J Pediatr 80* 858 1972
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ASYMPTOMATIC BACTERIURIA IN SCHOOLGIRLS

III Relation between Residual Urine Volume and Recurrence

U LINDBERG J BJURE S HAUGSTVEDT and U JODAL

From the Departments of Paediatrics Paediatric Surgery and Paediatric Clinical Physiology University of Goteborg Goteborg Sweden

ABSTRACT Lindberg U Bjure J Haugstvedt S and Jodal U (Departments of Paediatrics Paediatric Surgery and Paediatric Clinical Physiology University of Goteborg Goteborg Sweden) Asymptomatic bacteriuria in schoolgirls. III Relation between residual urine volume and recurrence Acta Paediatr Scand 64 437 1975.—Residual urine volume (RUV) has been measured in 70 schoolgirls with asymptomatic bacteriuria using [131] I hippuran The mean volume of residual urine was 23 7 ml More than 5 ml RUV the highest value found in 14 girls without bacteriuria or a history of urinary tract infection was found in 47 % of the patients The mean volume for residual urine in the control girls was 1 1 ml Among the 44 girls cured for their bacteriuria by a bladder wash out test or antibiotic treatment recurrence was significantly more common ($p<0\ 001$) in those with >5 ml in RUV (15/20 75 %) than in those with ≤5 ml (4/24 17 %) In 5 of 22 patients not treated the bacteriuria disappeared spontaneously during a one year observation period The RUV was <3 ml in 4 of these 5 girls While the presence of residual urine *per se* may not be the primary event leading to bacteriuria it may increase the risk of recurrences by facilitating bacterial growth and impairing the wash out of bacteria from the bladder on micturition

KEY WORDS Asymptomatic bacteriuria schoolgirls residual urine volume recurrence

It has been suggested that in addition to the possible importance of the immune mechanisms (6 9) failure to empty the bladder completely is of significance in urinary tract infection (UTI) (13) By using *in vitro* models simulating conditions of bacterial growth in the urinary tract it has been shown that the number of bacteria in the bladder depends on the bacterial growth rate the urine flow rate the frequency of urination and the residual urine volume (RUV) (12) These experimental findings may be of clinical significance since measurements of RUV using [131]I hippuran in women with symptomatic UTI showed that increased RUV was associated with difficulty in treating the infection (16)

In the present study we have used [131]I hippuran to measure RUV in schoolgirls with asymptomatic bacteriuria (ABU) as a part of a longitudinal study of the host–parasite relationship in UTI (10)

MATERIAL AND METHODS

The 70 patients were girls 7–15 years old with ABU i e with two consecutive positive nitrite tests in a school screening program two urine cultures with more than 100 000 bacteria/ml urine and the same type of bacteria in the two samples (10) All children appeared well and the physical examination did not reveal any abnormalities The control group consisted of 14 schoolgirls who had no history of urinary tract infection and a negative urine culture Seven of the 14 controls were treated for fractures 5 had been observed at the surgical ward for abdominal pain and 2 had had a glomerulonephritis

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MATERIAL AND METHODS

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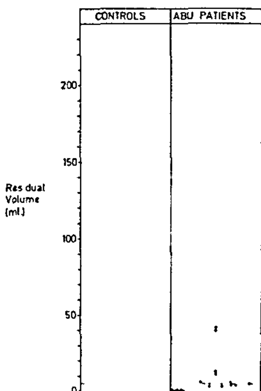


Fig 1 Residual urine volumes in 70 schoolgirls with asymptomatic bacteriuria and in 14 control girls

After emptying the bladder each subject was given 0 3 μCi/kg of [131]I hippuran intravenously and a renogram tracing was made After 2 hours during which period the subjects were not allowed to void the activity over the bladder area was measured by external counting in supine position before and shortly after voiding The background activity was measured over the precordium A scintillation counter with a sodium iodide crystal 2 5 cm in diameter was used for the external counting The distal internal diameter of the collimator was 5×10 cm and the skin–crystal distance was 12 cm Before the examination potassium iodide was given orally in order to block the thyroid uptake The residual urine was calculated from the formula

$$R_r = \frac{(C2 - B)\,V}{C1 - C2}$$

where
R_r = residual urine volume
C1 = activity over the bladder before voiding
C2 = activity over the bladder after voiding
B = background
V = volume voided

Without prior knowledge of the RUV the patients were allocated to three groups those who had (*a*) bladder wash out (*b*) treatment with nitrofurantoin or (*c*) no treatment

In 22 of the 70 girls a bladder washout was performed using a previously described (7) modification of the technique of Fairley et al (5) Eighteen of these 22 patients had a negative culture one day after the test Twenty six patients were treated successfully with nitrofurantoin 3 mg/kg/day for 10 days without a prior bladder washout Twenty two of the 70 patients were left untreated

Three 6 9 and 12 months after the initial detection a midstream specimen of urine from each girl was examined The criteria for persistence or recurrence was at least two consecutive urine cultures with ≥100 000 bacteria/ml and for a disappearance of bacteriuria at least two consecutive negative urine cultures

For *statistical evaluation* the chi square test was employed

RESULTS

Control group

The residual urine volume ranged between 0 and 5 ml with a mean of 1 1 ml for the 14 girls (Fig 1)

ABU patients

In the 70 patients RUVs between 0 and 238 ml were found with a mean of 23 7 ml (Fig 1) Thirty three girls (47%) had volumes >5 ml

Twelve of the 18 girls whose urine cleared after having a bladder washout did not have a recurrence during the following year Nine of these 12 girls had RUVs <5 ml and 3 had >5 ml (6 32 and 42 ml) All 6 patients with recurrence had RUVs >5 ml with values ranging between 10 and 74 ml (Fig 2) Of the 26 girls treated with 10 days of nitrofurantoin 13 did not get any recurrence during the one year of observation Eleven of these 13 patients had RUVs <5 ml and 2 had >5 ml (17 and 215 ml) Of the 13 patients with recurrence 9 had RUVs ranging between 7 and 238 ml four had ≤5 ml (Fig 2)

Of 44 patients cured for the bacteriuria (18 with a bladder washout 26 with nitrofurantoin) one or more recurrences were found in 19 patients and no recurrence for one year in 25 patients Fifteen of the 19 patients with recurrence had RUVs >5 ml and 20 of the 25 patients without recurrence had RUVs <5 ml There were significantly more recurrences in patients with >5 ml RUV than in those with <5 ml ($p<0\ 001$)

In the 19 patients with recurrences these occurred within 3 months in 17 girls and within 6 months in 2 girls Of the 22 patients not treated 10 had >5 ml RUV Five of the 22 untreated girls became spontaneously abacteriuric during one year The RUVs in these patients were 0 0 0 2 and 17 ml

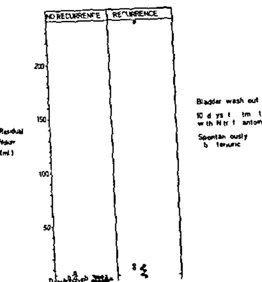


Fig 2 Residual urine volumes and recurrence in 44 girls cured of their bacteriuria by a bladder washout or by treatment with nitrofurantoin and in 5 girls with a spontaneous disappearance of bacteriuria

DISCUSSION

Since measurement of RUV by catheterization may be traumatic and introduce infection methods to measure residual volumes without catheterization are to be preferred Intravenous radiopaque dye can be used to detect residual urine radiographically (2) The method of Cotran & Kass involves an injection of phenolsulphonphthalein and 3 hours later the collection of two urine specimens at an interval of one hour (4) The use of ^{131}I-diodrast described by Murlow et al (11) may be employed even in the presence of reduced kidney function as can the similar method applied in the present study where ^{131}I hippuran was injected

Though free of some of the drawbacks of previous methods the estimation of RUV with ^{131}I hippuran has possible sources of error (1) Firstly an assessment of a tissue background radiation comparable to the one in the bladder region is important to the calculation of the RUV In adults the activity over the precordium (8 11) or umbilicus (1 15) has been measured A falsely high reading has been suspected over the precordium (14) In children we have found comparable tissue background activities over precordium lungs and thighs but higher values over the umbilicus (3) So in the present study the background activity over the precordium has been estimated to avoid a possible influence from the kidneys and residual urine in the bladder Secondly small amounts of ^{131}I hippuran continue to be excreted in the urine for some hours after intravenous injection even when the renal function is good and a period of at least 3 minutes is likely to elapse between voiding and the midpoint of external counting A small volume will be added to the residual urine in calculation This error has been estimated to 0 03–0 41 ml in adults (1)

Normal values for RUV in healthy children have been determined by von Steinert by catheterization of 280 healthy girls and 280 healthy boys 1–14 years old (17) The mean value for girls 7 years old was 3 1 ml and for girls 14 years old 3 8 ml which was somewhat more than the 1 1 ml found in 14 control girls in the present study If the urine production (0 77 ml/min in children 8–14 years) during the time the catheter is in the bladder is deducted from the values obtained after catheterization the results would probably be comparable In 9 healthy adult women Shand et al found <1 ml RUV using ^{131}I hippuran (16)

In the present study RUV >5 ml i e more than the highest RUV among the control girls was found in 47% of the 70 school girls with ABU After initial treatment recurrences were significantly more common among the girls with values >5 ml (15/20 i e 75%) than among those with ≤5 ml (4/24 i e 17%) A higher frequency of recurrences was also found by Shand et al in adult women with symptomatic UTI and increased RUV than in those without increased RUV (16)

Though the number of untreated patients is small it may be significant that among the 22 girls not treated there were 5 who became spontaneously abacteriuric during one year of observation and that 4 of these 5 children had RUV <3 ml

Although it is not possible from this study to say whether or not the presence of increased RUV is related to the pathogenesis of UTI or caused by the infection it is clear that its presence is related to the course of the disease

Further studies are necessary to explain the cause of increased residual urine volume and to find possible means to reduce it

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REFERENCES

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3 Bjure J Unpublished data
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7 Jodal U Lindberg U & Lincoln D Level diagnosis of symptomatic urinary tract infectious in childhood *Acta Paediatr Scand 64* 201 1975
8 Lindberg I F & Brandt N J Indirect determination of the volume of residual urine with ¹³¹I labeled hippuran *Acta Chir Scand 127* 675 1964
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13 O Grady F Path M C & Catell W R Kinetics of urinary tract infection II The bladder *Br J Urol 38* 156 1966
14 Rosenthall L Residual urine determination by roentgenographic and isotope means *Radiology 80* 454 1963
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EFFECT OF GLUCAGON ON BLOOD GLUCOSE HOMEOSTASIS IN INFANTS OF DIABETIC MOTHERS

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From the Department of Pediatrics University of Southern California School of Medicine and the Los Angeles County – USC Medical Center USA

ABSTRACT Wu P Y K Modanlou H and Karelitz M (Department of Pediatrics University of Southern California School of Medicine Los Angeles County University of Southern California Medical Center Los Angeles USA) Effect of glucagon on blood glucose homeostasis in infants of diabetic mothers Acta Paediatr Scand 64 441 1975.—Thirty infants of diabetic mothers (IDM) were randomly selected and divided into 3 groups of 10 babies each Group A were used as controls Group B received glucagon 300 μg/kg i m and Group C received glucagon 300 μg/kg i v at birth Hypoglycemia developed in 6 infants in Group A and 4 infants in Group B None of the infants in Group C had hypoglycemia Mean blood glucose was higher in Group C in the first 3 hours than Group A and higher in Group B in the first 1/2–1 hour I v glucagon 300 μg/kg when given in the first 15 minutes after birth prevented hypoglycemia in IDM in the first hours of life

KEY WORDS Infants of diabetic mothers glucagon blood glucose hypoglycemia

In infants of diabetic mothers (IDM) blood glucose concentrations fall rapidly in the immediate postnatal period reaching its lowest level between 1/2 and 4 hours (4 7 12 14 18 19)

The incidence of hypoglycemia has been reported to be as high as 58% in infants of insulin dependent mothers (20) Controversies exist with regard to the significance of this early hypoglycemia to the clinical course and future developmental disability in IDM (7 10 12 15 16 18) However the association of symptomatic hypoglycemia and cerebral damage has been documented by a number of investigators (1 5 9 17) In view of the potential risk of hypoglycemia it would appear reasonable to maintain blood glucose concentrations in IDM at normal levels

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Glucagon is known to activate adenyl cyclase which increase cyclic adenosine monophosphate the latter activates the phosphorylases to break down glycogen (2 3 11) In IDM with excess of liver glycogen glucagon by promoting glycogenolysis may assist in glucose homeostasis An earlier study by Cornblath et al (4) showed that glucagon when given to IDM between 30 minutes and 2 hours of age can evoke a hyperglycemic response

The present study was conducted in order to determine whether glucagon given within a few minutes after birth can effectively prevent hypoglycemia in IDM

METHODS

Thirty infants of insulin-dependent diabetic mothers were randomly selected and divided at birth into 3 groups

Group A Used as controls and did not receive any glucagon

Although it is not possible from this study to say whether or not the presence of increased RUV is related to the pathogenesis of UTI or caused by the infection it is clear that its presence is related to the course of the disease

Further studies are necessary to explain the cause of increased residual urine volume and to find possible means to reduce it

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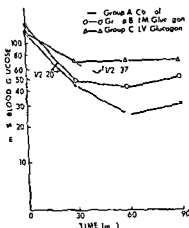


Fig 2 Apparent rate of fall in blood glucose in the 3 groups of infants

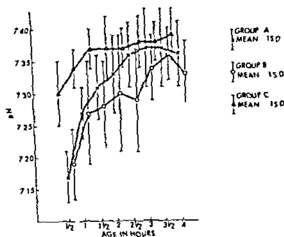


Fig 3 Changes in arterial and capillary blood pH in the 3 groups of infants during the first 4 hours

the blood glucose concentrations from the 3 groups of infants whose data are shown in Fig 1 Data is plotted semilogarithmically in the first 90 min after delivery and differences in the apparent rates of fall (disappearance) of glucose are compared In the first component 0–30 min the $t_{\frac{1}{2}}$ of Groups A and B were similar and is equal to 20 min while the $t_{\frac{1}{2}}$ of Group C was 37 min The K apparent was 3 4% per minute in the Groups A and B compared with 1 8% per minute in Group C

In the second component 30–60 min the $t_{\frac{1}{2}}$ of Group A was 35 min and Group B was 36 min while the K apparent was 1/8 and 1/9% per minute respectively

In the third component 60–90 min Groups A and B showed a parallel rise in blood glucose Group C showed no change in the second and third component

There were no significant differences in the mean arterial and capillary blood pH between Groups A and B (Fig 3) However Group C had significantly higher mean blood pH at ½ 1 and 1½ hours than Groups A and B ($p = < 0.05$)

The mean respiratory rate of Groups A and B were similar while Group C had significantly lower mean respiratory rate than Groups A and B ($p = < 0.05$) (Fig 4)

Heart rate and body temperatures were comparable in the 3 groups of infants

DISCUSSION

The incidence of hypoglycemia in the control infants in this study was comparable to that reported previously by Warner & Cornblath (20) Blood glucose concentration fell rapidly in the immediate postnatal period and reached its lowest level at 1 hour (Fig 1) Glucagon when given intravenously at birth exerted its action by raising blood glucose and thereby decreasing the apparent rate of fall of blood glucose within the first 30 min (Fig 2) In addition it

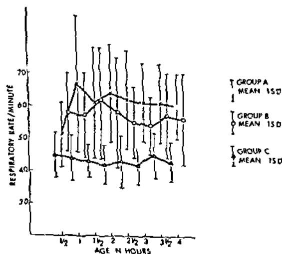


Fig 4 Changes in respiratory rate in the 3 groups of infants during the first 4 hours

Table 1 *Mean birth weight gestational age and sex distribution of infants*

Groups	No of infants	Delivery Vaginal	C section	Apgar 1 min	5 min	B W (g) $m \pm S D$	Gestational age (wks) $m \pm S D$	Sex M F
Control (Group A)	10	6	4	6 4±1 5	7 5±1 2	3 845±1 052	38 4±3 ?	6 4
i m Glucagon (Group B)	10	7	3	6 5±1 7	7 5±1 4	3 851±1 070	38 2±1 6	5 5
i v Glucagon* (Group C)	10	6	4	6 5±1 2	7 5±1 4	3476±841	38 7±1 1	5 5

* Dose of glucagon=300 μg/kg

Group B Received glucagon 300 μg/kg intramuscularly within 15 minutes after birth

Group C Received glucagon 300 μg/kg intravenously via umbilical vein within 15 minutes after birth

The dose 300 μg/kg was selected to avoid response differences associated with mode of delivery and maturity (18) The birth weight gestational age and sex distribution of the infants were comparable in the 3 groups as shown in Table 1 The distribution of the infants in the 3 groups according to the modified White s classification (19) of the classes of diabetes of their respective mothers is shown in Table 2

Infants received glucagon in the delivery suite Following this the infants were transferred to the special care unit and nursed in incubators Heart rate respiration rate rectal temperatures pH and blood gases and blood glucose were monitored sequentially at half hourly intervals during the first 4 hours and subsequently at 12 24 and 48 hours Infants developing hypoglycemia (20 mg/100 ml for pre term and 30 mg/100 ml for term infants) were treated with i v glucose and subsequent blood glucose values were excluded from this report Blood glucose was determined by glucose-oxidase peroxidase method (13) in neonatal research laboratory

RESULTS

Serial changes in the mean blood glucose con centration in the 3 groups of infants at 1/2 hourly intervals from birth to 4 hours of age are shown in Fig 1 The mean blood glucose in cord blood was similar in the 3 groups Mean blood glucose was significantly higher ($p=<0.01$) in the i v glucagon group (Group C) than in the control group (Group A) from 1/2 to 3 hours The i v glucagon group also had higher mean blood glucose than the i m glucagon group (Group B) from 1/2 to 1 hours Significant differences ($p=<0.05$) were also found in the blood glucose values between the i m (Group B) glucagon and control group (Group A) at 1 and 1½ hours Six out of 10 infants in Group A and 4 out of 10 infants in Group B developed hypoglycemia None of the infants in Group C had hypoglycemia In those infants that did not develop hypoglycemia blood glucose concentrations were similar in the 3 groups at 12 24 and 48 hours Fig 2 shows

Table 2 *Distribution of infants according to class of maternal diabetes*

Modified White s Classification (19)

Groups	Class B	Class C	Class D	Class F
Control (Group A)	6	2	2	0
i m Glucagon (Group B)	6	3	0	1
i v Glucagon (Group C)	4	5	1	0

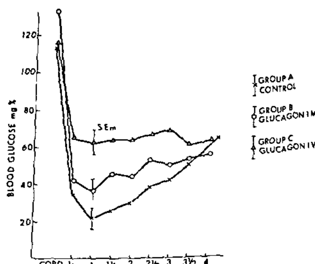


Fig 1 Mean blood glucose levels during the first 4 hours in the 3 groups of infants

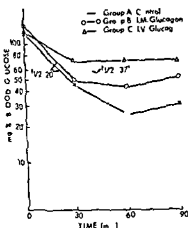


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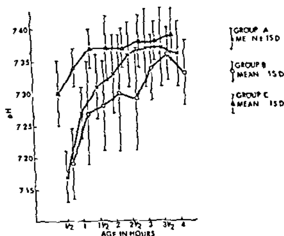


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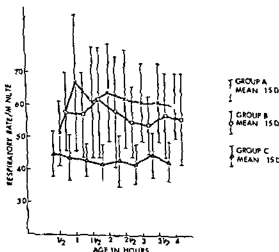


Fig 4 Changes in respiratory rate in the 3 groups of infants during the first 4 hours

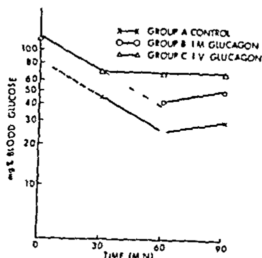


Fig 5 — calculated apparent rate of fall in blood glucose following birth drawn to demonstrate the similarity in the apparent rate of fall in blood glucose between the i v glucagon group in the first 30 min and control group during the second 30 min

also appeared to enable the infant to stabilize blood glucose earlier The effect of intramuscular glucagon was not evident prior to 30 min since the apparent rate of fall of blood glucose in this group of infants appeared to be similar to that of the control group (Fig 2) It was interesting to note that the pattern of change in blood glucose in the control group during the second period (i e 30–60 min) was found to be similar to that of the i v glucagon group in the first 30 min as shown by the dotted lines in Fig 5 This suggests that in the control group after 30 min endogenous regulatory mechanisms (possibly endogenous glucagon) were set into operation in response to the fall in blood glucose concentration in the first 30 min thus enabling 4 of the infants to raise their blood glucose spontaneously from 1½ to 4 hours (Fig 1) In the 6 infants that developed hypoglycemia presumably this endogenous regulatory mechanism was ineffective This finding lends support to Farquhar s hypothesis (8) that infants of diabetic mothers not only have hyperinsulinism but also have hypoglucagonism Thus they respond rapidly to i v glucagon but take some time to raise their blood glucose spontaneously from hypoglycemic levels McCann et al (15) demonstrated a significant relation between sequential changes of respiratory rate and blood glucose Our data on sequential measurement of blood pH and respiratory rate also indicate higher respiratory rate and lower blood pH in the two groups on infants (i e Groups A and B) who had lower blood glucose These differences in the blood pH and respiratory rate suggest that clinically the infants with the higher blood glucose concentrations appeared to be more stable

REFERENCES

1 Brown R J K & Wallis P G Hypoglycemia in newborn infant *Lancet I* 1278 1963
2 Burger M & Kramer H Primare Hyperglykamie und Glykogenverarmung der Leber als Folge intraportaler Insulininjektion nach Untersuchungen am Hund *Z Gesamte Exp Med 67* 441 1929
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5 Cornblath M Odell G B & Levin E Y Symptomatic neonatal hypoglycemia associated with toxemia of pregnancy *J Pediatr 55* 545 1959
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7 Farquhar J W The significance of hypoglycemia in the newborn infant of the diabetic woman *Arch Dis Child 31* 203 1956
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10 Hartman A F & Jandon J C Hypoglycemia *J Pediatr 11* 1 1959
11 Kibler R F & Meyers J D Responsiveness to the pancreatic hyperglycemic glycogen factor (HGF) as a test of liver function *Clin Res Proc 1* 109 1953
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13 Marks V An improved glucose oxidase method for determining blood C S F and urine glucose levels *Clin Chim Acta 4* 395 1959
14 McCann M L Chen D H Kangbak E B Kotchen J M [illegible] R E & Schwartz R Effects of

fructose on hypoglucosemia in infants of diabetic mothers *N Engl J Med 275* 7 1966
15 McCann M L Adam P A J Likly B F & Schwartz R Prevention of hypoglucosemia by fructose in infants of diabetic mothers *N Eng J Med 275* 8 1966
16 Miller H C & Ross R A Relation of hypoglycemia to symptoms observed in infants of diabetic mothers *J Pediatr 16* 473 1940
17 Neligan G A Robson E & Watson J Hypoglycemia in the newborn *Lancet I* 1?8? 1963
18 Pedersen J Bojsen Møller B & Poulson H Blood sugar in newborn infants of diabetic mothers *Acta Endocrinol (Kbh) 15* 33 1954
19 Pennoyer M M & Hartman A F Sr Management of infants born of diabetic mothers *Postgrad Med 18* 199 1955
?0 Warrner R A & Cornblath M Infants of gestational diabetic mothers *Am J Dis Child 117* 678 1969
21 White P Symposium on Diabetes Mellitus pregnancy complicating diabetes *Am J Med 7* 609 1949

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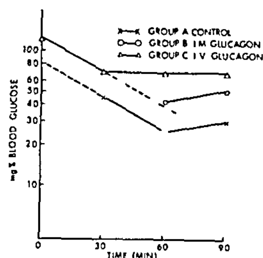


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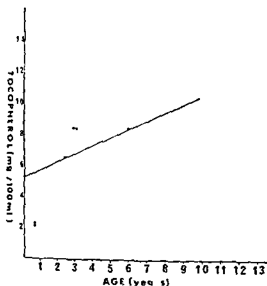


Fig 1 Plasma tocopherol values in the normal children

RESULTS

Plasma tocopherol values in normal infants and children are shown in Fig 1 and Table 1 In a series of 19 venous umbilical cord bloods from term infants the mean plasma tocopherol was 0 38 mg/100 ml (S D 0 1) The regression line $y=0\ 004x+0\ 52$ where y is the plasma tocopherol and x is the age in months is shown in Fig 1 This regression line covers the age range 4 months to 10 years ($r=0\ 55$ $p<0\ 001$) After the age of 10 years there appears to be no tendency for a further rise in the plasma tocopherol The plasma tocopherol values of the abnormalities are shown in Table 2 One patient with a β lipoproteinaemia aged 14

Table 1 *Plasma tocopherol values in normal children*

Age (years)	No of patients	Plasma tocopherol (mg/100 ml) Mean±S D
Birth	19	0 38±0 1
4/1 –1	13	0 45±0 _3
1–2	8	0 65±0 18
?–4	1	0 73±0 ?4
4–6	13	0 75±0 ?4
6–8	9	0 30±0 22
8–10	14	0 9?±0 28
10–14	11	0 83±0 18

Table 2 *Plasma tocopherol values in abnormal patients*

Diagnosis	Age (years)	Plasma tocopherol (mg/100 ml)	*t* test vs normals
Group 1			
Giardiasis	11/12	0 39	
Giardiasis	15/1?	0 45	
Giardiasis	?1/1?	0 06	
Giardiasis	??/1?	0 61	$p>05$
Giardiasis	2	0 85	
Giardiasis	?	0 6_	
Giardiasis	4	0 80	
Group 2			
Cystic Fibrosis	3/1?	0 43	
Cystic Fibrosis	4/1?	0 11	
Cystic Fibrosis	4/1?	0 00	
Cystic Fibrosis	9/12	0 08	
Cystic Fibrosis	16/1?	0 08	$p<001$
Cystic Fibrosis	3	0 _8	
Cystic Fibrosis	3	0 ?5	
Cystic Fibrosis	4	0 16	
Cystic Fibrosis	6	0 74	
Group 3			
Coeliac Disease	5/1?	0 00	
Coeliac Disease	6/1?	0 08	
Coeliac Disease	6/12	0 00	
Coeliac Disease	11/1_	0 9	
Coeliac Disease	1	0 30	
Coeliac Disease	17/1?	0 31	$p<001$
Coeliac Disease	3	0 ?6	
Coeliac Disease	5	0 ?6	
Coeliac Disease	8	0 11	
Coeliac Disease	9	0 9	
Coeliac Disease	10	0 35	

years had a plasma tocopherol of 0 1 mg/100 ml while receiving an oral tocopherol supplement of 3 g daily The regression line was used to compare the plasma tocopherol values of the normal patients with those of the abnormals using Student s *t* test It can be seen from Table 2 that there is no significant difference between the patients in group 1 and the normals but that the difference between groups 2 and 3 and the normals was highly significant

DISCUSSION

The data presented confirm that the plasma tocopherol rises with age and show a similar trend to that of a previous study (2) The most rapid rise occurs during the first two years of

PLASMA TOCOPHEROL IN INFANTS AND CHILDREN

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From the Department of Child Health University of Dundee Dundee Scotland

ABSTRACT McWhirter, W R (Dept of Child Health Ninewells Hospital Dundee Scotland) Plasma tocopherol in infants and children Acta Paediatr Scand 64 446 1975 —A study has been made of plasma tocopherol concentrations in normal children and in children with intestinal abnormalities A positive correlation between plasma tocopherol and age is shown between the ages of 4 months and 10 years Most patients with cystic fibrosis or coeliac disease were found to have markedly reduced plasma tocopherol concentrations in comparison with the normal children of similar age

KEY WORDS Tocopherol steatorrhoea

Tocopherol (Vitamin E) is a normal constituent of the diet and is found in relatively high concentrations in foods such as cereals and some leafy vegetables as well as in human colostrum Its main function after absorption is probably as an anti oxidant it is thought to play a part in inhibiting the oxidation of cell membrane lipids by hydrogen peroxide produced by the cell s metabolism Deficiency in infancy has been shown to cause haemolytic anaemia (11) and it is likely that the retinal and neurological abnormalities of a β lipoproteinaemia (10) as well as the ceroid pigmentation in the muscles of patients with cystic fibrosis (7) are related to Vitamin E deficiency

That Vitamin E a fat soluble vitamin is poorly absorbed in patients with steatorrhoea has been demonstrated previously (3 9 13) There is however comparatively little detailed information on the plasma tocopherol levels found in normal children at different ages Cord blood concentrations are considerably below those found in normal adults in whom the mean value is around 1 mg/100 ml (4) It is clear therefore that the plasma tocopherol must rise with age and this has been shown in some earlier studies (3 14) The present study was undertaken to confirm the trend in plasma tocopherol concentrations in children between the ages of 4 months and 14 years who did not have malabsorption and to compare them with those from children with various forms of intestinal abnormality

MATERIALS AND METHODS

The normal group consisted of children attending with minor complaints such as enuresis or upper respiratory tract infection who had no clinical features suggestive of malabsorption but who required a venepuncture as part of their normal investigations The abnormal patients included seven with Giardia lamblia infestation (group 1) nine with cystic fibrosis (group 2) eleven with coeliac disease (group 3) and one with a β lipoproteinaemia. Plasma tocopherol was estimated in duplicate on specimens of venous heparinised plasma by the method of Martinek (8) using 0 5 ml plasma The S E M of the duplicates was 0 017 mg/100 ml

The patients were not fasting at the time the specimens were taken It was considered that fasting was not necessary since it has been shown that plasma tocopherol reaches a maximum 9 hours after ingestion of a tocopherol load and only falls gradually thereafter (1)

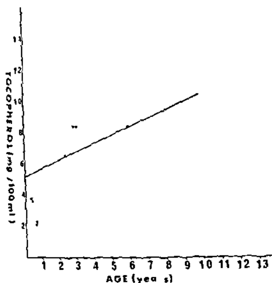


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6–8	9	0.90±0.2[illegible]
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Giardiasis	2	0.62	
Giardiasis	4	0.80	
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Cystic Fibrosis	3/12	0.43	
Cystic Fibrosis	4/12	0.11	
Cystic Fibrosis	4/12	0.00	
Cystic Fibrosis	9/12	0.08	
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Cystic Fibrosis	3	0.28	
Cystic Fibrosis	3	0.25	
Cystic Fibrosis	4	0.16	
Cystic Fibrosis	6	0.74	
Group 3			
Coeliac Disease	5/12	0.00	
Coeliac Disease	6/12	0.08	
Coeliac Disease	6/12	0.00	
Coeliac Disease	11/12	0.29	
Coeliac Disease	1	0.30	
Coeliac Disease	17/12	0.31	$p<.001$
Coeliac Disease	3	0.26	
Coeliac Disease	5	0.26	
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COAGULATION CHANGES ASSOCIATED WITH A HIGH HAEMATOCRIT IN THE NEWBORN INFANT

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ABSTRACT Rivers R P A (Departments of Paediatrics and Clinical Haematology University College Hospital Medical School London W C 1 England) Coagulation Changes Associated with a High Haematocrit in the Newborn Infant Acta Paediatr Scand 64 449 1975.—Sequential blood coagulation studies were performed on samples from three newborn infants with venous haematocrit values of over 65% Combinations of thrombocytopaenia circulating fibrin monomer and evidence of intravascular thromboplastic activity were found Reduction of the haematocrit value by partial exchange transfusion in each infant was followed by improvement of the abnormal coagulation findings Some possible mechanisms for the origin of the coagulation abnormalities are considered

KEY WORDS Coagulation haematocrit newborn viscosity

Although high haematocrit values in the newborn infant are known to be associated with a number of clinical observations including plethora (31) cyanosis and cardio-respiratory abnormalities (11 15 32 41 46) convulsions (15 41 46) lethargy (3) vomiting (2) jaundice (3 5 25) priapism and hypoglycaemia (20) a retinopathy (30) and a thrombotic tendency (4 37) we have found no reports of the coagulation abnormalities such as we have recently observed in three neonates This association is of considerable interest in relation to the bleeding diathesis in the newborn infant which is regarded by some authors to result from a variable degree of excessive clotting factor and platelet consumption leading in the fully developed form to disseminated intravascular coagulation (DIC) It is well known that a rise in haematocrit increases blood viscosity greatly reducing flow through the microcirculation a condition likely to favour coagulation Hence the association of a high haematocrit and evidence of coagulation in these three infants with haematocrit values of over 65% is of particular interest

Abbreviations

DIC disseminated intravascular coagulation
PTT partial thromboplastin time
MPTT mixture partial thromboplastin time
TCaT thrombin calcium time
FM fibrin monomer
SDPST serial dilution protamine sulfate titre
fdp fibrin degradation products
FDP fibrinogen degradation products

LABORATORY METHODS

Blood for the coagulation studies was collected from a freshly inserted umbilical vein catheter or from a peripheral blood vessel as indicated in Tables 1 and 2 The blood was diluted in 1/10 volume of 3 8% w/v trisodium citrate to which epsilon amino caproic acid had been added to inhibit *in vitro* fibrinogenolysis The kaolin activated partial thromboplastin time (PTT) using Bell & Alton Platelet Substitute (Diagnostic Reagents Thame Oxon) and 10 minutes activation was performed on a control normal adult plasma on the test plasma and on a 1 1 mixture of control and test plasmas hereafter referred to as the mixture partial thromboplastin time (MPTT)

The thrombin time was performed using bovine thrombin solution (containing 50 units/ml) diluted in 0 025

life It is therefore important when referring to plasma tocopherol values in children to relate these to the normal values for the ages con cerned It has been shown by Horwitt et al (6) that the serum tocopherol correlates fairly well with serum lipids it is therefore possible that some of the rise in tocopherol levels with age can be attributed to the rise in plasma lipids

Tocopherol deficiency is usually defined in adults as a plasma level of less than 0 5 mg/100 ml since patients with levels in this range usually show increased hydrogen peroxide haemolysis (2) It will be apparent therefore that many children under the age of 5 years have tocopherol deficiency by adult standards although clinical manifestations of deficiency such as haemolytic anaemia thrombocytosis and oedema have been reported only in infants during the first 2 or 3 months of life (5 12) There is no evidence to suggest that this physiological deficiency in pre school children is of clinical importance It is not cur rent practice to give healthy children toco pherol along with other vitamins and in the light of presently available information there would appear to be no need to do so

ACKNOWLEDGEMENT

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REFERENCES

1 Filer L J Wright S W Manning M P & Mason K E Absorption of α tocopherol and tocopherol esters by premature and full term infants and children in health and disease *Pediatrics 8* 328 1949
2 Gordon H H Nitowsky H M Tildon J T & Levin S Studies of tocopherol deficiency in infants and children *Pediatrics 21* 673 1958
3 Harries J T & Muller D P R Absorption of different doses of fat soluble and water miscible preparations of vitamin E in children with cystic fibrosis *Arch Dis Child 46* 341 1971
4 Harris P L Hickman K C D Jensen J L & Spiers T D Survey of blood plasma levels of vi min A carotene ascorbic acid and tocopherols of persons in an area of endemic malnutrition *Am J Public Health 36* 155 1946
5 Hassan H Hashim S A van Itallie T B & Seb ell W H Syndrome in premature infants associated with low plasma vitamin E levels and high polyunsatura ed fatty acid diet *Am J Clin Nutr 19* 147 1966
6 Horwitt M K Harvey C C Dahm C H & Sea. cy M T Relationship between tocopherol and serum lipid levels for determination of nutritional adequacy *Ann NY Acad Sci 203* 223 1972
7 Kerner I & Goldbloom R B Investigations of tocopherol deficiency in infancy and childhood stu h s of ceroid pigment deposition *Am J Dis Child 99* 597 1960
8 Martinek R G Method for the determina ion of vitamin E (total tocopherols) in serum *Clin Chem 10* 1078 1964
9 Muller D P R & Harries J T Vitamin E studies in children with malabsorption *Biochem J 112* 28P 1969
10 Muller D P R Harries J T & Lloyd J K Vitamin E therapy in a β lipoproteinaemia *Arch Dis Child 45* 715 1970
11 Oski F A & Barness L A Vitamin E deficiency a previously unrecognised cause of haemolytic an aemia in the premature infant *J Pediatr 70* 211 1967
12 Ritchie J H Fish M B McMasters V & Gross man M Edema and haemolytic anaemia in prematur infants *N Engl J Med 279* 1185 1968
13 Underwood B A & Denning C R Blood and live concentrations of vitamins A and E in children wit cystic fibrosis of the pancreas *Pediatr Res 6* 26 197
14 Wright S W Filer L J & Mason K E Vitamin blood levels in premature and full term infants *Pediatrics 7* 386 1951

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Age (hours mins)	Site								
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72 15	CFV	47	–	–	–	–	–	–	–
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37 00	UV	68	–	–	–	–	–	–	–
72 40	FV	67	36	52	+2	–	–	190	60
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* In this laboratory for control adults and neonates of the gestations and ages of Cases 1 and 2

M calcium chloride to give a concentration of 4 5–5 0 units/ml equal volumes of thrombin-calcium and plasma being used (TCaT) (final thrombin concentration = 2 25–2 5 units/ml)

Platelets were counted by light microscopy (10) fibrinogen levels determined by an optical density technique using thrombin (8) fibrin monomers (F M) and fibrin degradation products (fdp) were detected by the serial dilution protamine sulphate titre (SDPST) (18 35) and fibrin degradation product (fdp) and fibrinogen degradation product (FDP) complexes were measured on Arvin treated plasma using sensitized latex particles (Latex Thrombo Wellcotest Wellcome Reagents Limited Wellcome Research Laboratories Beckenham Kent England B R 3 3 B S) a quantitative slide agglutination test

The haematocrit was determined in duplicate using a microcapillary centrifuge (Hawksley & Sons Ltd London) on venous blood collected into EDTA the specimens being centrifuged for 10 min IgA determinations were made to detect recent materno-foetal transfusion as a cause of the high haematocrit but none was detected (23)

CASE REPORTS

Case 1

A normal female birth weight 3 37 kg gestation 37 weeks born by a normal vertex delivery to a diabetic mother on dietary control At 6 hours of age the infant was noted to be plethoric and to have areas of cyanosis on both feet she was tachypnoeic with a frequency of 60/min her haematocrit value was 66% Following partial exchange with 50 ml 0 9 NaCl at 10 hours of age her haematocrit value fell to 51% and the respiratory frequency decreased to 40/min The total serum bilirubin level reached 6 5 mg/100 ml on the fourth day

Case 2

A normal female the first of twins born by normal vertex delivery following a normal pregnancy of 35 weeks gestation birth weight 1 96 kg Her respiratory frequency was 50–70/min and at 32 hours of age her haematocrit was 80% Following partial blood exchange with 50 ml 0 45% NaCL at 33 hours of age her respiratory frequency decreased to 45/min and the haematocrit to 68% The total serum bilirubin level reached 8 mg/100 ml at 36 hours

Her twin sister birth weight 1 97 kg had a haematocrit value of 57% at 24 hours of age and her total serum bilirubin concentration reached 7 8 mg/100 ml on the third day Her respiratory frequency did not exceed 45/min

Case 3

A normal male birth weight 2 40 kg the second twin born to a primigravid Rhesus negative mother without anti rhesus antibodies Labour was induced following the development of mild pre-eclamptic toxaemia at 36 weeks gestation Both deliveries were normal This twin became hypoglycaemic at 17 hours of age and by 61 hours appeared plethoric The haematocrit was 72% *following* partial blood exchange at 64 hours with 30 ml 0 18% NaCl in 10% dextrose the haematocrit fell to 58% The total serum bilirubin concentration reached 12 mg/100 ml on the fourth day

Platelets (×10 /mm³)	Serial dilution protamine sulphate titre	Latex Thrombo-Wellcotest (FDP/fdp) (mg/100 ml)
150–400	age >72 hrs ≤1/5 ≤4 0 <72 hrs ≤1/10	
0	1/20	<0 8
153 178 175	1/20 – 1/5	<0 8
112	1/20	<1 6
143 187	– 1/20 –	<0 8

RESULTS

The results of sequential blood coagulation studies are listed in Tables 1 and 2 Normal values derived from sequential studies on 20 infants of similar gestational and age ranges to Cases 1 and 2 are shown at the top of Table 1 and from 10 infants of similar gestational and age range to Case 3 in Table 2 The mixing of equal volumes of control adult and normal neonatal plasma in the PTT test never resulted in a time which was shorter than the adult control PTT nor was the normal neonate s TCaT ever shorter than the adult control TCaT utilizing the same freshly prepared thrombin calcium solution

In all three cases we found thrombocyto paenia and circulating fibrin monomer (FM) as evidenced by a positive SDPST in as sociation with a negative test result for fibrin and fibrinogen degradation products (Latex Thrombo-Wellcotest) The shortened TCaT is probably due to the presence of fibrinogen–FM complexes Addition of thrombin to such complexes in the presence of calcium (TCaT) results in faster fibrin clot for mation than when added to non FM containing plasma (1 42)

There is also evidence of circulating clot promoting activity as indicated by a shortening of the MPTT compared with the PTT of the adult control plasma

The lack of any consistent change in plasma fibrinogen level following treatment is due to the dilution of this protein in a greater vol ume of plasma associated with the fall in haematocrit A more meaningful expression of the amount of fibrinogen available for clot for mation may be derived from the whole blood fibrinogen level (19) where the rise following treatment is seen in all three cases

In Case 1 (Fig 1) thrombocytopaenia was present in association with a MPTT which was shorter than the PTT of either control or test sample The positive SDPST is indicative of circulating FM complexed in this case with fibrinogen since no fdp or FDP were detectable with the latex test The TCaT of the infant s plasma was also abnormal being shorter than that of the control

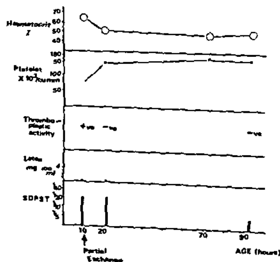


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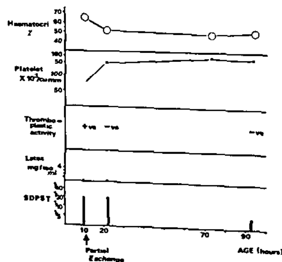


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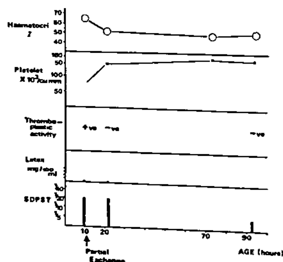


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Platelets were counted by light microscopy (10) fibrinogen levels determined by an optical density technique using thrombin (8) fibrin monomers (FM) and fibrin degradation products (fdp) were detected by the serial dilution protamine sulphate titre (SDPST) (18 35) and fibrin degradation product (fdp) and fibrinogen degradation product (FDP) complexes were measured on Arvin treated plasma using sensitized latex particles (Latex Thrombo Wellcotest Wellcome Reagents Limited Wellcome Research Laboratories Beckenham Kent England B R 3 3 B S) a quantitative slide agglutination test

The haematocrit was determined in duplicate using a microcapillary centrifuge (Hawksley & Sons Ltd London) on venous blood collected into EDTA the specimens being centrifuged for 10 min IgA determinations were made to detect recent materno foetal transfusion as a cause of the high haematocrit but none was detected (23)

CASE REPORTS

Case 1

A normal female birth weight 3 37 kg gestation 37 weeks born by a normal vertex delivery to a diabetic mother on dietary control At 6 hours of age the infant was noted to be plethoric and to have areas of cyanosis on both feet she was tachypnoeic with a frequency of 60/min her haematocrit value was 66% Following partial exchange with 50 ml 0 9 NaCl at 10 hours of age her haematocrit value fell to 51% and the respiratory frequency decreased to 40/min The total serum bilirubin level reached 6 5 mg/100 ml on the fourth day

Case 2

A normal female the first of twins born by normal vertex delivery following a normal pregnancy of 35 weeks gestation birth weight 1 96 kg Her respiratory frequency was 50–70/min and at 32 hours of age her haematocrit was 80% Following partial blood exchange with 50 ml 0 45% NaCL at 33 hours of age her respiratory frequency decreased to 45/min and the haematocrit to 68% The total serum bilirubin level reached 8 mg/100 ml at 36 hours

Her twin sister birth weight 1 97 kg had a haematocrit value of 57% at 24 hours of age and her total serum bilirubin concentration reached 7 8 mg/100 ml on the third day Her respiratory frequency did not exceed 45/min

Case 3

A normal male birth weight 2 40 kg the second twin born to a primigravid Rhesus negative mother without anti rhesus antibodies Labour was induced following the development of mild pre-eclamptic toxaemia at 36 weeks gestation Both deliveries were normal This twin became hypoglycaemic at 17 hours of age and by 61 hours appeared plethoric The haematocrit was 72% following partial blood exchange at 64 hours with 30 ml 0 18% NaCl in 10% dextrose the haematocrit fell to 58% The total serum bilirubin concentration reached 12 mg/100 ml on the fourth day

Platelets $\times 10^3/mm^3$	Serial dilution protamine sulphate titre	Latex Thrombo-Wellcotest (FDP/fdp) (mg/100 ml)
150–400	age >72 hrs ≤1/5 <72 hrs ≤1/10	≤4 0
97	1/20	<0 8
95	1/20	–
	1/20	–
140	1/10	–
182	1/10	<0 8
187	1/5	–
1 0	1/5	–
63	1/5	–

of anticoagulant often result in clotting of the sample possibly due to poor mixing in the viscous mass of erythrocytes In order to prevent such *in vitro* coagulation the volume of anticoagulant was not reduced in this study and in Cases 2 and 3 this might explain the longer PTT measurements before in comparison with after exchange However any such dilutional effect would result in a reduction in level of thromboplastic activity and in underestimation of the level of fibrin monomer The fibrinogen level was corrected for anticoagulant dilution

The SDPST methodology has been extensively studied by Gurewich et al (18) and as compared with the protamine sulphate test (27) is semiquantitative in the detection of fibrin monomer and early fdp The site of sampling does not affect the result provided that a two syringe technique is employed in sampling from a freshly inserted saline flushed umbilical catheter and that peripheral samples are obtained quickly and cleanly This may be noted from the results of Case 3 where FV and UV samples gave a similar result for the SDPST prior to partial exchange transfusion Confirmatory evidence for the presence of FM as compared with fdp was provided by the abnormally short TCaT in Cases 1 and 3

In the neonatal period thrombocytopenia has occasionally been recorded in association with a high haematocrit (17 34 46) The clinical conditions in childhood in which coagulation changes associated with a high haematocrit have been described are mostly those with high haematocrit values in association with cyanotic congenital heart disease (12 19 21 40) where the mechanism of production of the thrombocytopaenia when present has been disputed (13 22) and variable improvements in platelet count and coagulation status following venesection and replacement therapy have been described (19 21 40)

In our cases there is no evidence of DIC as defined by a combination of low fibrinogen level thrombocytopaenia and raised level of fdp in the neonate with DIC it is usual to find elevated levels of fdp and these were undetectable (<0 8 mg/100 ml) in 2 of our infants and very low in the third nor can the absence of fdp be attributed to a low fibrinolytic potential since the newborn infant has been shown not to be deficient in this regard (14) so that the absence of fdp makes established DIC as a cause of the thrombocytopaenia most unlikely However our findings are compatible with an ongoing low grade intravascular coagulation process

Origin of coagulation changes

The abnormal coagulation findings in these 3 infants do not of themselves indicate by what mechanism the changes might have been initiated Both platelets and erythrocytes might however be involved Palmer (39) has shown that the aggregates of erythrocytes which form at high haematocrit values tend to occupy the central region of small vessels and that platelets are forced to flow close to the vessel walls the region of highest shear rate These shearing forces may be capable of causing damage to

Table 2 *Results of coagulation studies in new born twins one of whom had an elevated haematocrit value*

UV umbilical vein CFV cubital fossa vein FV femoral vein FA femoral artery

		Hct (%)	PTT (sec) Adult control	PTT (sec) Neonate	Mixture 1 1 Neonate and Adult Control	TCaT (sec) Adult control	TCaT (sec) Neonate	Fibrinogen (mg/100 ml plasma)	Whole blood fibrinogen (mg/100 ml blood)
Normal values* Case 3	No of infants 10	<65	32–42	38–50	Adult control time +1 0–9 0	10 5–16 5	Adult control time +0 5–1 0	170–450	40–160
Age (hours mins)	Site								
Case 3									
61 50	FV	72	35	71	+2	17 5	−1 0	220	60
63 30	UV	70	36	70	+2	17	−1 0	–	–
Partial exchange 30 ml withdrawn 1/5 N saline 10% dextrose replacement									
69 00	FA	58	–	–	–	–	–	–	–
80 00	FV	56	–	–	–	–	–	–	–
87 20	FV	55	40	51	+2	14	−0 5	220	100
10 days	FV	55	36	45	+2	–	–	235	105
Twin of Case 3									
61 50	FV	54	35	76	+9	17 5	+0 5	230	105
10 days	FV	46	36	39	+1	–	–	270	145

* In this laboratory for control adults and neonates of the gestation and age of Case 3

Following partial exchange the MPTT became normal the TCaT and SDPST remaining abnormal probably due to the continued circulation of previously formed fibrin monomer The platelet count had risen following exchange

In Case 2 there was mild thrombocytopaenia in association with similar results in the PTT and MPTT estimations to those in Case 1 both the SDPST and latex tests were positive but following partial exchange the persistence of a positive SDPST with a negative latex test implied that fibrin monomer was principally giving rise to the positive SDPST rather than fdp A small rise in platelet level was noted which had further increased by 95 hours

In Case 3 the findings were similar to Case 1 with the exception that both the test PTT and MPTT estimations were longer than the control PTT, although the MPTT was shorter than that measured in the infant s twin sister whose results are given for comparison Following exchange there was a rise in platelet count persistence of the level of fibrin monomer and shortening of the PTT The level of fibrin monomer had fallen by 11 hours after the partial exchange

DISCUSSION

Association of high haematocrit and coagulation changes

The findings in our three infants suggest that clotting factor activation intravascular fibrin monomer formation and platelet consumption had occurred Attention has often been drawn to the technical problems involved in the investigation of coagulation status in the presence of a high haematocrit due to the increase in anticoagulant/plasma ratio resulting from the low plasma volume of the sample an effect which cannot be allowed for in any simple way except in the case of an actual concentration measurement Attempts to reduce the volume

Platelets $\times 10^3$/mm³	Serial dilution protamine sulphate titre	Latex Thrombo-Wellcotest (FDP/fdp) (mg/100 ml)
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–	1/20	–
140	1/10	–
18[illegible]	1/10	<0 8
187	1/5	–
170	1/5	–
263	1/5	–

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platelets as can the local acidosis caused by tissue hypoxia in reduced flow states (7) Such platelets may then be capable of activating the clotting mechanism via the intrinsic system (43)

Erythrocyte membrane damage induced by mechanical and physicochemical factors including hypoxia and acidosis can result in contact factor activation and actual liberation of material with coagulant properties may occur (6 28) The increased mechanical fragility of erythrocytes in the newborn infant (16 44) would facilitate such release Evidence in support of the possibility of erythrocyte damage in the presence of a high haematocrit in the neonatal period comes from the commonly associated finding of jaundice and microscopic observation of red blood cell fragmentation (17)

Whatever the primary mechanism involved the sluggish flow would favour intravascular thrombin generation due to the delayed removal of platelet products and activated clotting factor complexes Any depression in levels of the naturally occurring inhibitors of these activated factors would also increase the likelihood of intravascular coagulation In this connection levels of anti thrombin III and anti activated factor X have been shown to be reduced in some neonates (29) Further with regard to platelet aggregation this can also be induced by the action of intermediate products of fibrinogen digestion by thrombin (24 26 36) and such a mechanism might well operate under conditions of stasis permitting prolonged platelet contact with such fibrin monomer/fibrinogen complexes

Treatment

Dramatic clinical improvement following venesection and replacement infusion (4 11 17 45 46) has been described in the neonate with polycythaemia In the cases followed here such therapy resulted in improvement of the abnormal coagulation findings and of the clinical symptoms when present Whether treatment of a high haematocrit value in the absence of clinical symptomatology is necessary remains to be determined however when the coagulation abnormalities of thrombocytopaenia with an increased level of FM exist in view of the risk of thrombotic complications and uncertain cerebral consequences of impaired oxygen transfer (9) treatment would seem advisable

Venesection with replacement of an equal volume of fluid carried out as an exchange procedure in 5–10 ml increments appears to be satisfactory the volume to be removed being given by the formula

$$\frac{\text{Blood volume} \times (\text{Observed Hct} - \text{Desired Hct})}{\text{Observed Hct}} \quad (38)$$

Although it remains uncertain what the ideal replacement fluid in this situation is whether saline plasma or albumin in view of the likely low renal output in the neonate with hyperviscous blood the use of low molecular weight dextrans (40000 mol wt) as replacement fluid should probably be avoided since a very viscerous urine will be produced and cases of renal failure with anuria due to renal tubular cell deposition of such dextrans have been described (33)

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REFERENCES

1 Arnesen H Studies in thrombin clotting time II Influence of fibrin degradation products *Scand J Haemat 10* 291 1973
2 Baum R S Hyperviscous blood and perinatal pathology *Pediatr Res 1* 288 1967
3 Becker A H & Glass H Twin to twin transfusion syndrome *Am J Dis Child 106* 624 1963
4 Benfield D G Lubbe R J & Sutherland J M Therapeutic consequences of partial exchange transfusion in symptomatic polycythaemic neonates Program American Pediatric Society *Pediatr Res 6* 406/146 1972

5 Bergstedt J Monozygoti twins one with high erythrocyte values and jaundice the other with anaemia neonatorum and no jaundice *Acta Paediatr Scand 46* 701 1957

6 Bradlow B A Liberation of material with platelet like coagulant properties from intact red cells and particularly from reticulocytes *Br J Haematol 7* 476 1961

7 Broersma R J Bullemer G D & Mammen E F Acidosis induced disseminated intravascular microthrombosis and its dissolution by streptokinase *Thromb Diath Haemorrh 24* 55 1970

8 Burmester H B C Aulton K & Horsfield G I Evaluation of a rapid method for the determination of plasma fibrinogen *J Clin Pathol 23* 43 1970

9 Crowell J W Ford R G & Lewsi V M Oxygen transport in hemorrhagic shock as a function of the hematocrit ratio *Am J Physiol 196* 1033 1959

10 Dacie J V *Practical haematology* J & A Churchill London 1968 p 72

11 Danks D M & Stevens L H Neonatal respiratory distress associated with a high haematocrit reading *Lancet II* 499 1964

1 Dennis L H Stewart J L & Conrad M E Heparin treatment of hemorrhagic diathesis in cyanotic congenital heart disease *Lancet I* 1088 1967

13 Ekert H Gilchrist G S Stanton R & Hamond D Haemostasis in cyanotic congenital heart disease *J Pediatr 76* 221 1970

14 Ekelund H Hedner U & Nilsson I M Fibrinolysis in newborns *Acta Paediatr Scand 59* 33 1970

15 Gatti R A Muster A J Cole R B & Paul M H Neonatal polycythaemia with transient cyanosis and cardio respiratory abnormalities *J Pediatr* 69 1063 1966

16 Goldbloom R B Fischer E Reinhold J & Hsia D Y Studies on the mechanical fragility of erythrocytes I Normal values for infants and children *Blood 8* 165 1953

17 Gross G P Hathaway W E & McGaughey H R Hyperviscosity in the neonate *J Pediatr 8*? 1004 1973

18 Gurevich V Lipinski B & Lipinska I A comparative study of precipitation and paracoagulation by protamine sulfate and ethanol gelation tests *Thromb Res* ? 539 1973

19 Hartmann R C A hemorrhagic disorder occurring in patients with cyanotic congenital heart disease *Johns Hopkins Hospital Bull tin 91* 49 195

0 Humbert J R Abelson H Hathaway W E & Battaglia F C Polycythaemia in small for gestational age infants *J Pediatr 75* 812 1969

21 Jackson D P Hemorrhagic diathesis in patients with cyanotic congenital heart disease pre-operative management *Ann N Y Acad Sci 115* 235 1964

22 Johnson C A Abildgaard C F & Schulman I Absence of coagulation abnormalities with cyanotic congenital heart disease *Lancet II* 660 1968

3 Kontras S B Polycythaemia and hyperviscosity syndromes in infants and children *Pediatr Clin North*

24 Kopec M Z Wegrzynowicz A Z Budzynski Z S Latallo B Lipinski B & Kowalski E Interaction of fibrinogen degradation products (FDP) with platelets *Exp Biol Med 3* 73 1968

25 Kresky B Transplacental transfusion syndrome *Clin Paediatr 3* 600 1964

26 Larrieu M J Rigollot C & Kubisz P Platelet aggregation induced by soluble fibrin monomer complexes *Life Sci 9 Pt 2* 1111 1970

27 Lipinski B & Worowski K Detection of soluble fibrin monomer complexes in blood by means of protamine sulphate test *Thromb Diath Haemorrh 20* 44 1968

28 Lopas H & Birndorf W I Haemolysis and intravascular coagulation due to incompatible red cell transfusion in isoimmunised monkeys *Br J Haematol 21* 399 1971

29 Mahasandana C & Hathaway W E Circulating anticoagulants in the newborn relation to hypercoagulability and the idiopathic respiratory distress syndrome *Pediatr Res 7* 670 1973

30 McCormick A Q Segal S & Pirie G E A new retinopathy in low birthweight infants Program American Pediatric Society *Pediatr Res 6* 413 1972

31 Michael A F & Mauer A M Maternal fetal transfusion as a cause of plethora in the neonatal period *Pediatrics 28* 458 1961

32 Minkowski A Le retentissement cardiaque de la polycythemie neonatale (jumeaux) et post natale (enfants uniques) Biol *Neonate 4* 61 196?

33 Morgan T O Little J M & Evans W A Renal failure associated with low molecular weight dextran infusion *Br Med J II* 737 1966

34 Naiman J L & Schlackman N *Transient* thrombocytopaenia in the neonatal polycythaemia syndrome *Program Soc Paediatr Res p 241* 1971

35 Niewiarowski S & Gurewich V *Laboratory* identification of intravascular coagulation *J Lab Clin Med 77* 665 1971

36 Niewiarowski S Regoeczi E Stewart G J Senyi A F & Mustard J F Platelet interaction with polymerising fibrin *J Clin Invest 51* 685 1972

37 Oppenheimer E H & Esterly J R Thrombosis in newborn comparison between infants of diabetic and non-diabetic mothers *J Pediatr 67* 549 1965

38 Oski F A & Naiman J L *Hematologic Problems in the Newborn* W B Saunders & Co Philadelphia 1972 pp 80-81

39 Palmer A A Platelet and leucocyte skimming 4th Europ Conf Microcirc Cambridge 1966 *Bibl Anat 9* 300 1967

40 Paton B C von Kaulla K N von Kaulla E & Rosenkrantz J G Safe definitive correction of polycythaemic cyanotic tetralogy of Fallot after gradual pre-operative correction of coagulation *Circulation* Suppl *III* 18 1966

41 Raynaud E J Godeneche P Gaulme J & Malpuech G Les polyglobulies neonatales Etude clinique et physiopathologique de 44 observations *Ann Pediatr 48* 2889 1972

4? Rivers R P A Unpublished data

43 Sciffman S Rapaport S I & Chong M M Y

Platelets and initiation of clotting *Br J Haematol 24* 633 1973
44 Sjolin S The resistance of red cells in vitro *Acta Paediatr Scand 43* 390 1954
45 Sommer A & Kontras S B Studies of blood viscosity in the normal newborn *Biol Neonate 17* 441 1971
46 Wood J L Plethora in the newborn associated with cyanosis and convulsions *J Pediatr 54* 143 1959

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Acta Pædiatr Scand 64 457–463 1975

GLUCOSE TOLERANCE AND INSULIN SECRETION IN VERY SMALL BABIES

A CSER and R D G MILNER

From the Department of Child Health University of Manchester England

ABSTRACT Cser A and Milner R D G (Department of Child Health University of Manchester St Mary s Hospital Manchester M13 OJH England) Glucose tolerance and insulin secretion in very small babies Acta Paediatr Scand 64 457 1975.—Nineteen exchange transfusions were performed via the umbilical artery using blood preserved with acid citrate and dextrose in 8 infants of 34–40 weeks gestation (larger infants) and 9 very small infants of 26–33 weeks gestational age The plasma glucose rise which was similar in both groups stimulated insulin secretion from the larger infants but not the very small infants No significant differences occurred between the groups in the fall in mean free fatty acid levels or increase in growth hormone secretion Following transfusion there was a sharp rise in mean plasma insulin concentration in the larger infants and a smaller rise in the very small infants but the rate of glucose disappearance was greater in the very small infants A highly significant positive correlation was found between the maximum post transfusion plasma insulin and the birth weight of the infants Plasma glucose levels of less than 30 mg/100 ml occurred in 2 larger and 5 very small infants during the first 3 hours after transfusion One infant of birth weight 0 98 kg received four transfusions in 2 where he received ACD blood via the umbilical artery or vein insulin secretion was not stimulated but in the other 2 in which glucagon or arginine was added to the ACD donor blood insulin secretion was stimulated Feeding practice should take account of the fact that although very small infants secrete less insulin than larger infants during exchange transfusion they are more likely to become hypoglycaemic in the immediate post transfusion period

KEY WORDS Insulin secretion glucose homeostasis very small infants

Use has been made of exchange transfusions to study the interaction of various metabolites and hormones in newborn infants (6 7 8 10 11) The approach suffers from the disadvantage that experimental variables such as postnatal age and time from the last meal are difficult to control and that most observations are made on infants with haemolytic disease of the newborn who have abnormal islets of Langerhans (12) On the other hand this approach uses waste blood collected during a therapeutic procedure to make observations which would otherwise be ethically difficult if not impossible

In this report the handling of glucose by very premature infants has been studied both during and after exchange transfusion The very small infant has been shown to respond to transfusion with less insulin secretion than larger infants but in spite of this to have a relatively rapid rate of glucose disappearance and an increased tendency to hypoglycaemia The results also demonstrate how the insulin secretory response to glucose increases with birth weight and postnatal age

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Exchange transfusions were carried out via the umbilical artery on two groups of infants as described previously (?) and blood was collected during and for 3 hours after trans

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Table 2 *Total amount of metabolites or hormones infused and removed during exchange transfusion*

Hormone or metabolite	Amount infused or removed/kg body weight (mean ±S E M)		
	In	Out	Balance
Very small infants (12)			
Glucose (mg)	444±45	137±12	+306±36
FFA (μmol)	4 0±5 8	74 3±4 4	−32 3±4 5
Insulin (mU)	2 13±0 53	04±0 41	+0 71±0 51
GH (μg)	0 11±0 03	4 18±0 54	−4 07±0 51
Larger infants (9)			
Glucose (mg)	4 6±45	157±14	+ 69±39
FFA (μmol)	31 4±4 8	97 8±15 5	−66 3±12 1
Insulin (mU)	1 69±0 23	2 99±0 41	−1 31±0 48
GH (μg)	0 13±0 03	6 47±1 24	−6 34±1 31

Number of observations in parentheses
Level of significance for comparison with larger infants $p<0\ 05$

was similar being 67 hours in the very small infants and 76 hours in the larger infants The volume of blood exchanged was also similar when expressed as a function of body weight varying between 144 and 221 ml/kg in the larger infants and 144 and 215 ml/kg in the very small infants Blood samples were collected after infusion of 100 200 300 and 400 ml in the larger infants and after 50 100 150 and 200 ml in the very small infants In 2 infants of birth weights 700 and 980 g blood samples were collected after infusion of 40 80 1 0 160 and 200 ml blood At various times after the transfusion 1 or 3 ml blood samples were collected from very small and larger infants respectively

RESULTS

During transfusion

At the start of transfusion the mean concentration of hormones and metabolites in the donor blood used for larger or very small infants was similar as were the concentrations in the babies themselves with the exception of the mean

Table 3 *Plasma metabolite and hormone concentrations in two infants weighing 0 70 and 0 98 kg during different types of exchange transfusion*

Infant and type of transfusion	Donor	0 ml	40 ml	80 ml	120 ml	160 ml	200 ml
0 70 kg Artery ACD at 49 hours							
Glucose (mg/100 ml)	430	25	87	106	90	–	–
FFA (μmol/l)	1 250	1 800	1 010	800	750	–	–
Insulin (μU/ml)	27	4	7	6	6		
0 98 kg Artery ACD at 32 hours							
Glucose (mg/100 ml)	570	152	168	174	166	160	202
FFA (μmol/l)	800	1 100	1 100	1 0 0	1 250	800	1 100
Insulin (μU/ml)	25	11	14	19	20	21	24
GH (ng/ml)	0 6	22	23	19	20	20	20
0 98 kg Artery ACD+glucagon at 55 hours							
Glucose (mg/100 ml)	465	–	158	04	220	224	235
FFA (μmol/l)	300	600	900	1 000	1 000	860	800
Insulin (μU/ml)	16	15	20	17	21	19	24
GH (ng/ml)	0 6	32	23	17	18	25	30
0 98 kg Vein ACD at 80 hours							
Glucose (mg/100 ml)	433	77	135	149	152	159	164
FFA (μmol/l)	200	2 600	1 4 0	1 300	960	1 00	700
Insulin (μU/ml)	15	3	5	9	10	11	17
GH (ng/ml)	1	12	13	15	2	23	27
0 98 kg Vein+arginine at 104 hours							
Glucose (mg/100 ml)	440	57	112	136	156	158	161
FFA (μmol/l)	600	1 400	1 680	1 200	950	950	600
Insulin (μU/ml)	11	9	14	30	38	35	0
GH (ng/ml)	1	23	17	13	10	9	12
Arginine (μmol/l)	400	34	31	43	61	49	63

Table 1 *Plasma metabolite and hormone concentrations in very small and larger infants during exchange transfusion (Mean ±S E M)*

	Donor	0 ml	50 ml	100 ml	150 ml	200 ml
Very small infants						
Glucose (mg/100 ml)	470±42 (*10*)	76±12 (*10*)	130±10 (*10*)	145±9 (*10*)	158±14 (*10*)	169±15 (*10*)
FFA (μmol/l)	417±57 (*10*)	806±80 (*10*) **	821±92 (*10*)	731±77 (*10*)	583±60 (*10*)	639±66 (*10*)
Insulin (μU/ml)	22±5 (*10*)	14±3 (*10*)	16±4 (*10*)	22±5 (*10*)	19±3 (*10*)	24±5 (*10*)
GH (ng/ml)	1.3±0.2 (9)	44±8 (9)	45±8 (9)	48±7 (9)	48±5 (9)	43±7 (9)
Larger infants	Donor	0 ml	100 ml	200 ml	300 ml	400 ml
Glucose (mg/100 ml)	520±31 (9)	71±8 (9)	127±14 (9)	149±5 (9)	165±5 (9)	175±12 (9)
FFA (μmol/l)	392±67 (9)	1 376±115 (9)	1 033±127 (9)	885±96 (9)	809±93 (9)	710±104 (9)
Insulin (μU/ml)	22±4 (9)	14±2 (9)	23±4 (9)	29±3 (9)	34±7 (9)	31±6 (9)
GH (ng/ml)	1.4±0.4 (9)	35±5 (9)	46±10 (9)	56±15 (9)	59±13 (9)	88±19 (9)

Number of observations shown in parentheses
Level of significance for comparison with larger infants $p<0.001$

fusion for the measurement of plasma glucose free fatty acid (FFA) insulin and growth hormone (GH) (1, 8) In one infant two transfusions were performed via the umbilical vein these are described separately All transfusions were performed with semi packed blood preserved with acid citrate and dextrose (ACD) In one transfusion 40 μg glucagon (Eli Lilly Ltd) was added to 300 ml donor blood and in another 17.4 mg L arginine hydrochloride (kindly prepared by Servier Laboratories) was added to 400 ml blood and plasma arginine levels during this transfusion were measured by cation exchange chromatography using an automated gradient elution device

Ten transfusions were performed in 8 infants with a mean gestational age of 37 weeks (range 34–40) and a mean birth weight of 3.02 kg (range 2.12–3.84) these are referred to as the larger infants Thirteen transfusions were performed on 9 preterm infants of mean gestational age of 29 weeks (range 26–33) and mean birth weight 1.20 kg (0.70–1.56) these are referred to as the very small infants The two groups differed in that all of the larger infants but only two of the very small infants suffered from haemolytic disease of the newborn Most transfusions in the very small infants were because of jaundice of prematurity complicated by bruising or infection The mean postnatal age at transfusion

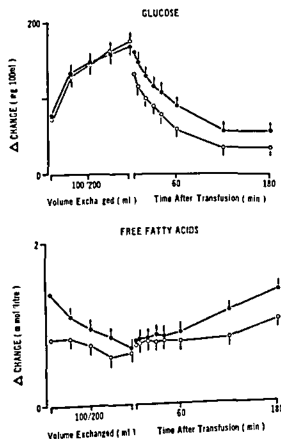


Fig 1 Changes in mean ±S E M concentration of glucose insulin and free fatty acids during and for 180 min following exchange transfusion The larger infants are shown by closed symbols very small infants by open circles The scale for volume transfused is 0–400 ml for the larger infants and 0–200 ml for the very small infants

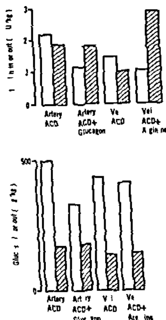


Fig 2 Total amounts of glucose (below) and insulin (above) infused (open columns) and removed (hatched columns) during four exchange transfusions of an infant weighing 0 98 kg The route of transfusion and nature of the donor blood is shown beneath each pair of columns

and a net insulin loss (Table 4 Fig 2) The addition of arginine to the donor blood in an amount sufficient to cause a rise in plasma arginine within physiological limits during a venous transfusion resulted in the greatest rise in plasma insulin and negative insulin balance of the four transfusions

After transfusion

In the first hour after transfusion the mean plasma insulin level of the larger infants rose appreciably and significantly more than that of the very small infants (Table 5 Fig 1) The rise in mean plasma insulin level indicated that both groups responded to exchange transfusion with increased insulin release From 60 to 180 min after transfusion there was no significant difference in the mean plasma insulin concentration between the two groups Despite lower plasma insulin levels the mean blood glucose levels were lower and fell faster in the very small infants in the post transfusion period The mean (±S E M) rate of glucose disappearance during the first 60 min of the larger infants was 1 33±0 20 which did not differ significantly from that of the very small infants 1 47±0 19 During the second and third hours post transfusion minimum plasma glucose levels of 8 14 14 21 and 26 mg/100 ml were recorded in five of the very small infants whereas only two of the larger infants had plasma glucose levels of less than 30 mg/100 ml (22 and 22 mg/100 ml) The mean plasma FFA levels in both groups remained steady and similar for the first hour after transfusion and then began to rise The mean plasma GH level in the

60 min	1?0 min	180 min
ʼ8±8 (10)	33±5 (10)	33±6 (10)
758±8? (9)	799±103 (9)	1 0 0±116 (8)
6±6 (10)	?9±7 (10)	0±6 (9)
31±5 (9)	38±7 (9)	?5±6 (8)
90±14 (9)	53±8 (9)	55±9 (9)
891±179 (9)	1 143±185 (9)	1 491±147 (9)
4?±13 (9)	34±13 (9)	2?±4 (9)
74± 3 (9)	71±27 (9)	39±16 (9)

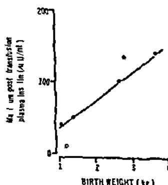


Fig 3 Scatterdiagram of maximum plasma insulin level in the post transfusion period in 19 infants as a *function of* birth weight Infants with haemolytic disease are shown in closed circles others in open circles The straight line describes the relationship maximum insulin=38 birth weight−1 7 correlation coefficient 0 769 $p<0\ 001$

Table 4 *Insulin/glucose ratios of infants having more than one exchange transfusion*

Birth weight (kg)	Gestational age (weeks)	Postnatal age (h) and insulin/glucose ratio (mU/g)		
		Transfusion 1	Transfusion 2	Transfusion 3
1 08	28	49 0	92 6 9	–
1 38	32	26 0 9	74 0 4	100 6 0
2 12	35	5 5 9	13 7 1	–
3 06	37	–	50 2 7	74 6 7

All infants suffered from haemolytic disease of the newborn The insulin/glucose ratio was calculated as the net amount of insulin removed divided by the net amount of glucose infused

plasma FFA level which was significantly higher in the very small infants (Table 1) During transfusion there was no significant difference between the groups in the mean plasma concentration of hormone or metabolite when appropriate points were compared (Table 1) Analysis of the changes in plasma concentration (Fig 1) revealed that for an almost identical rise in mean plasma glucose concentration there was a smaller rise of mean plasma insulin and fall of mean plasma FFA levels respectively in the very small infants

In the calculation of the total amounts of hormone and metabolite infused and withdrawn transfusions in the 2 infants weighing 0 98 and 0 70 kg were included in the group of very small infants Each group had a similar net positive balance of glucose and negative balance of GH (Table 2) The glucose infusion caused a significant loss of insulin in the larger but not in the very small infants The net loss of FFA was significantly greater in the larger infants indicating that the greater fall in mean plasma FFA concentration during transfusion was due only in part to hyperglycaemia and hyperinsulinaemia stimulating lipogenesis and in part due to a washing out of FFA from the infant In 4 infants who had more than one transfusion the insulin/glucose ratio calculated as the amount of insulin removed from the infant per gram of glucose infused rose with postnatal age (Table 3) It is noteworthy that similar insulin/glucose ratios were observed in all these infants by the fifth day of life

Transfusion via the umbilical artery with ACD blood did not stimulate a rise in plasma insulin concentration above that in the donor blood in the two lightest infants studied (Table 4) nor was there a net loss of insulin (Fig 2) One of the two needed four transfusions and the opportunity was taken to change the transfusion conditions so that each transfusion provided a different insulinogenic stimulus Transfusion via the artery or vein failed to cause a significant rise in plasma insulin concentration above that in the donor blood or in net insulin loss from the infant but when glucagon was added to the donor blood for an arterial transfusion there was a modest rise in plasma insulin

Table 5 *Plasma metabolite and hormone concentrations in very small and larger infants afte exchange transfusion (Mean ±S E M)*

	5 min	10 min	20 min	30 min	40 min
Very small infants					
Glucose (mg/100 ml)	132±14 (*10*)	117±11 (*10*)	100±9 (*10*)	90±9 (*10*)	78±9 (*10*)
FFA (μmol/l)	745±52 (*8*)	755±77 (9)	801±87 (9)	760±75 (9)	773±94 (9)
Insulin (μU/ml)	30±7 (9)	34±8 (*10*)	35±8 (*10*)*	30±6 (*10*)*	30±7 (*10*)
GH (ng/ml)	39±8 (9)	33±6 (9)	29±6 (9)	33±7 (9)	34±7 (9)
Larger infants					
Glucose (mg/100 ml)	164±19 (9)	150±19 (9)	132±18 (9)	118±19 (9)	109±19 (9)
FFA (μmol/l)	749±145 (9)	766±140 (9)	801±156 (9)	831±170 (9)	811±151 (9)
Insulin (μU/ml)	53±14 (9)	64±10 (9)	68±13 (9)	63±10 (9)	73±17 (9)
GH (ng/ml)	75±17 (9)	77±19 (9)	69±18 (9)	73±22 (9)	56±15 (9)

Number of observations shown in parentheses Level of significance for comparison with larger infants $p<0\ 05$

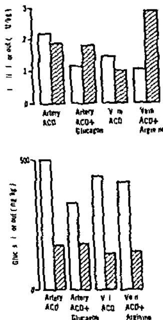


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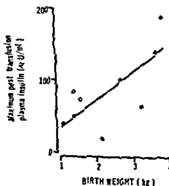


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Number of observations shown in parentheses Level of significance for comparison with larger infants *$p<0.05$

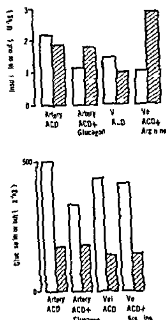


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60 min	120 min	180 min
58±8 (*10*)	33±5 (*10*)	33±6 (*10*)
7[illegible]8±8 (9)	799±103 (9)	1 0 0±116 (8)
[illegible]6±6 (*10*)	[illegible]9±7 (*10*)	0±6 (*9*)
31±5 (9)	38±7 (9)	35±6 (8)
90±14 (9)	53±8 (9)	55±9 (9)
891±179 (9)	1 143±183 (*9*)	1 491±147 (9)
4[illegible]±13 (9)	34±13 (9)	[illegible]±4 (9)
74±[illegible]3 (9)	71±27 (9)	39±16 (*9*)

After transfusion

In the first hour after transfusion the mean plasma insulin level of the larger infants rose appreciably and significantly more than that of the very small infants (Table 5 Fig 1) The rise in mean plasma insulin level indicated that both groups responded to exchange transfusion with increased insulin release From 60 to 180 min after transfusion there was no significant dif ference in the mean plasma insulin concentra tion between the two groups Despite lower plasma insulin levels the mean blood glucose levels were lower and fell faster in the very small infants in the post transfusion period The mean (±S E M) rate of glucose disap pearance during the first 60 min of the larger infants was 1 33±0 20 which did not differ significantly from that of the very small infants 1 47±0 19 During the second and third hours post transfusion minimum plasma glucose levels of 8 14 14 21 and 26 mg/100 ml were recorded in five of the very small infants whereas only two of the larger infants had plasma glucose levels of less than 30 mg/100 ml (22 and 22 mg/100 ml) The mean plasma FFA levels in both groups remained steady and simi lar for the first hour after transfusion and then began to rise The mean plasma GH level in the

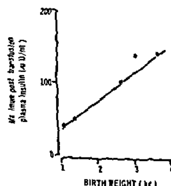


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very small infants remained steady after transfusion whereas that of the larger infants fell in the third hour to pretransfusion levels

The time at which the maximum plasma insulin level was observed always occurred in the post transfusion period but varied considerably being between 5 and 180 min post transfusion in the very small infants and between 5 and 60 min in the larger infants When the maximum post transfusion plasma insulin concentration was plotted as a scatterdiagram against the birth weight of the infant a positive correlation was observed (Fig 3)

DISCUSSION

The umbilical artery was chosen as the route for transfusion in this study because earlier work (2) had shown that transfusions performed via the vein caused more variable and higher plasma insulin levels Furthermore the arterial plasma insulin levels were of interest as these reflect the insulin concentration at most of the body s tissues Since the volume of blood transfused in an infant is mainly decided by the body weight of the baby it was logical to express all amounts infused or withdrawn as a function of weight and to sample blood from the very small infants at different points in the transfusion to the larger infants The chosen sampling points in the very small infants were endorsed by the fact that the mean plasma glucose concentrations in the two groups were almost identical Each group may thus be considered to have had an equivalent glucose infusion to which the very small infants failed to respond with a significant rise in plasma insulin It may be argued that this difference was because all nine of the transfusions in larger infants were performed for rhesus incompatibility of which hyperinsulinism is a characteristic (5) whereas only five of the 10 transfusions in very small infants were performed because of haemolytic disease Against this argument can be set the observation that no distinction could be made in the transfusions of very small infants when they were subdivided according to diagnosis (see Fig 3)

Inspection of the plasma insulin concentrations during transfusion gives the impression that the very small infants made no response to the glucose infusion That this was not so is seen in the plasma insulin levels after transfusion Only 2 of the 7 very small infants both of birth weights 1 16 kg and transfused for hyperbilirubinaemia of prematurity and bruising failed to show a rise of plasma insulin level at any time The larger infants all had their highest plasma insulin level in the first post transfusion hour whereas the very small infants were more variable three having their highest plasma insulin 120 min after the transfusion ended This delay in the response to glucose stimulation is characteristic of the immature β cell (4) Other evidence of increasing β cell maturity with advancing development was the positive correlation between maximum plasma insulin levels and birth weight

Some indication of the postnatal development of the insulin secretory response to glucose was given by those infants who had more than one transfusion The 4 infants contrasted markedly in birth weight and gestational age but each showed increased insulin secretion per gram of glucose infused with succeeding transfusions and by the fifth day of life all had a similar insulin/glucose ratio

The results of the present study agree in general with the previous report from this laboratory (7) in which the response of a more mature group of preterm infants transfused with ACD blood via the umbilical vein was compared with that of term infants An apparent difference is the larger rise in plasma GH levels and significantly greater net GH loss ($p<0.025$) noted in the preterm infants in that study compared with the very small infants in the present study Since transfusion of larger infants via the artery causes more GH secretion than transfusion via the vein (2) the route of transfusion is unlikely to explain the difference The possibility that the smaller GH secretion

noted in the present study is a function of gestational age awaits further investigation

The relatively rapid rates of glucose disappearance after transfusion and greater incidence of hypoglycaemia in the preterm group endorse earlier conclusions regarding the instability of glucose homeostasis in infants of this gestational age (3) Lack of data would reduce the investigator to speculation on the cause for this brittle glycaemia but the clinical message is clear In the management of very small babies extra care must be paid to the dangers of both hyper and hypoglycaemia

ACKNOWLEDGEMENTS

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REFERENCES

1 Cser A & Milner R D G Metabolic and hormonal changes during and after exchange transfusion with heparinized or ACD blood *Arch Dis Child 49* 940 1974
2 — Metabolic and hormonal consequences of exchange transfusion via the umbilical artery or vein *Biol Neonate* in press 1975
3 Dweck H S & Cassady G Glucose intolerance in infants of very low birth weight I Incidence of hyperglycemia in infants of birthweights 1100 grams or less *Pediatrics 53* 189 1974
4 Grasso S Personal communication 1974
5 Milner R D G Growth and development of the endocrine glands—endocrine pancreas *In* J A Davis & J Dobbing (eds) *Scientific Foundations of Paediatrics* W Heinemann Ltd London 1974 p 507
6 Milner R D G Chouksey S K & Assan R Metabolic and hormonal effects of glucagon infusion in erythroblastotic infants *Arch Dis Child 48* 885 1973
7 Milner R D G Fekete M & Assan R Glucagon insulin and growth hormone response to exchange transfusion in premature and term infants *Arch Dis Child 47* 186 1972
8 Milner R D G Fekete M Assan R & Hodge J S Effect of glucose on plasma glucagon growth hormone and insulin in exchange transfusion *Arch Dis Child 47* 179 1972
9 Milner R D G Fekete M Hodge J S & Assan R Influence of donor blood temperature on metabolic and hormonal changes during exchange transfusion *Arch Dis Child 47* 933 1972
10 Schiff D Aranda J V Chan G Colle E & Stern L Metabolic effects of exchange transfusions I Effect of citrated and of heparinized blood on glucose nonesterified fatty acids 2-(4 hydroxybenzeneazo) benzoic binding and insulin *J Pediatr 78* 603 1971
11 Schiff D Aranda J V Colle E & Stern L Metabolic effects of exchange transfusion II Delayed hypoglycemia following exchange transfusion with citrated blood *J Pediatr 79* 589 1971
12 Van Assche F A De Gasparo M & Renaer M The endocrine pancreas in erythroblastosis foetalis *Biol Neonate 15* 176 1970

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Some indication of the postnatal develop ment of the insulin secretory response to glu cose was given by those infants who had mor than one transfusion The 4 infants contraste markedly in birth weight and gestational ag but each showed increased insulin secretio per gram of glucose infused with succeedin transfusions and by the fifth day of life all had a similar insulin/glucose ratio

The results of the present study agree in general with the previous report from this laboratory (7) in which the response of a more mature group of preterm infants transfused with ACD blood via the umbilical vein was compared with that of term infants An apparent difference is the larger rise in plasma GH levels and significantly greater net GH loss ($p<0.025$) noted in the preterm infants in that study compared with the very small infants in the present study Since transfusion of larger infants via the artery causes more GH secretion than transfusion via the vein (2) the route of transfusion is unlikely to explain the difference The possibility that the smaller GH secretion

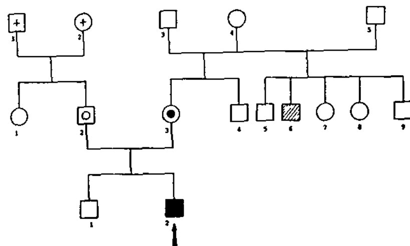


Fig 1 Pedigree of family 1

■ Clinical symptoms of hyperammonemia with proved OCT deficiency

◸ OCT deficiency without clinical symptoms

□ Normal OCT activity

◨ Unexplained death

Fig 1) died of encephalopathy at age 2 years one sister (II₇ Fig 1) also suffers from headaches

The proband displayed no particular symptoms during the neonatal period and infancy He surmounted several illnesses without difficulty reduction of a fractured humerus under general anesthesia in 1964 measles in January 1965 rubella in March 1965 In May 1965 he was hospitalized for diarrhea and acute dehydration in a pre comatose state which resolved without sequelae Between the ages of 4 and 8 years he presented 5 or 6 episodes of asthenia with vomiting transient jaundice and ketonuria persisting for 24 to 48 hours diagnosed as ketonemic vomiting At age 8 years the child had a normal psychomotor development attended school and was a good student

Symptoms appeared suddenly at the age of 8 years 2

Table 1 *Plasma and urine amino acids patient number 1*

	Plasma (µmoles/100 ml)		Urines (µmoles/24 h)	
	Patient	Control	Patient	Control
Taurine	5 3	7 4–21 6	22 5	60–969
Aspartic acid	4 6	0 5–2	11 25	0
Threonine	23	11 4–33 5	40 5	35–?48
Serine	15 8	1? 2–24 2	55 5	155–540
Glutamic acid	1 ?	2–10 7		0
Proline	15 8	10–27		0
Glycine	39	2? 4–51 4	172	160–1 420
α NH₂ butyr acid	2 7	0 8–4 9		
Valine	11.5	8–?4 6	Traces	15–50
1/? Cystine	Traces	2–14	Traces	40–160
Methionine	7 2	0 9–4 1	Traces	20–95
Isoleucine	4	2 7–9 4	Traces	18–56
Leucine	9 9	4 7–10 9	11 25	27–83
Tyrosine	13 5	4 2–9 9	20	42–169
Phenylalanine	13 2	4 2–11	10	24–106
Ornithine	9 7	4 9–15 1	20	0
Lysine	72	11 4–?6 9	41 25	70–640
Histidine	15 4	4 9–11 4	65	360–1 280
Arginine	11 9	? 2–8 8	Traces	0
α-alanine	65.5	23 6–41	195	12–440
Glutamine	118	15 3–89	35	14 7–84 7
α-NH₂ adipic acid	Traces	0	0	0

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HEREDITARY ORNITHINE TRANSCARBAMYLASE DEFICIENCY

Report of Two Male Cases with Residual Enzymatic Activity

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J A LEJEUNE and P MOZZICONACCI

From the Clinique Medicale Infantile Hopital des Enfants Malades the Laboratoire du Pr C Polonovski Hopital Trousseau Paris and the Laboratoire du Pr Lemonnier Hopital du Kremlin Bicetre Le Kremlin Bicetre France

ABSTRACT Saudubray, J M Cathelineau L, Laugier J M Charpentier C Lejeune J A and Mozziconacci P (Clinique Médicale Infantile Hopital des Enfants-Malades Paris Laboratoire du Pr C Polonovski Hopital Trousseau Paris and Laboratoire du Pr Lemonnier Hopital du Kremlin Bicetre Le Kremlin Bicetre France) Hereditary ornithine transcarbamylase deficiency Report of two male cases with residual enzymatic activity Acta Paediatr Scand 64 464 1975—The authors report two male cases of liver ornithine carbamyl transferase deficiency In one the disease occurred at 8 years of age with hyperammonemic coma leading to death in 48 hours In the second case symptoms appeared on the sixth day of life but the outcome was favorable The child is normal at 15 months In both cases there was a residual 6–10% OCT activity These observations are similar to two other male cases in the literature and are different from the male neonatal fatal form in which the deficiency is virtually total They underline the genetically heterogeneous nature of OCT deficiencies and the fact that in this X transmitted trait hemizygotes can preserve a functional enzymatic activity compatible with life

KEY WORDS Hyperammonemia ornithine transcarbamylase X chromosome hepatic metabolism inborn errors

Hereditary ornithine transcarbamylase (OCT) deficiency was first described by Russell in 1962 (17) in two females first cousins presenting as chronic hyperammoniemia Since then approximately 25 cases have been published half of which were males Clinical biochemical and genetic studies have allowed various authors to postulate a sex linked transmission of the disease (3, 4, 11 20 23)

With regard to the mode of transmission Short et al (23) and various American authors (3 11, 20) have noted that hemizygote boys always have an OCT activity which is virtually nil and that they are affected by a lethal neonatal form of the disease while the mother and the daughter carriers have an enzymatic activity with variable clinical manifestations We report here two male cases in which the clinical and biochemical symptomatology is quite different from the fatal neonatal form in that they occurred later in life and displayed a significant persistent enzymatic activity One of these cases has already been referred to briefly in a previous article (5)

CASE HISTORIES

Case I

Joel M born Dec 14 1963 was the second of two children The other brother age 11 is in good health The father aged 42 is in good health The mother age 36 suffers from migraine and frequent episodes of nausea Among the mother s siblings 4 males and 2 females one brother (II,

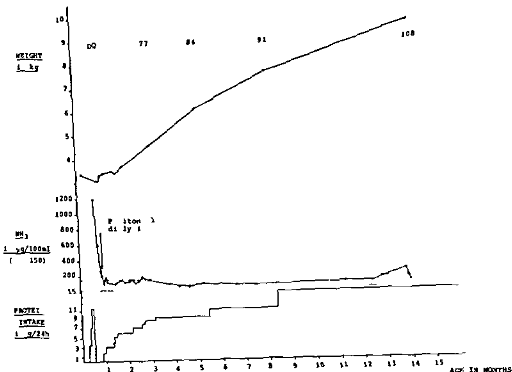


Fig 3 Patient no 2 Clinical course

tography showed a low excretion of all amino acids including glutamine (Table 1) The test for orotic acid (semiquantitative method of Rogers & Porter) (16) was strongly positive The test for methylmalonic acid was negative (method of Giorgio & Plaut) (9)

En ymatic assays

The entire liver was removed immediately following death and frozen at −80 C The urea cycle enzymes were studied as previously described (6 7 8) There existed a severe OCT deficiency as measured under standard conditions (residual activity equal to 10% of normal) associated with a definite but less marked carbamyl phosphate synthetase deficiency (approximately 25% of normal) Argino succinase and arginase activities were not decreased (Table 2) There was no modification of the K_m of OCT for one or the other substrate K_m carbamyl phosphate $0\ 31 \times 10$ M (control $0\ 40-0\ 70 \times 10^{3}$ M) K_m ornithine $0\ 44 \times 10$ M (control $0\ 35-0\ 57 \times 10^{3}$ M)

Study of the pa ents

Both mother and father were subjected to a protein loading test as well as needle biopsy of the liver for enzymatic studies The protein loading test (ingestion of 2 g of protein/kg) showed that both the father and the mother have a definite increase in their ammonia level at 4 hours following the meal (increase from 100 at 200 μg/100 ml in the father and from 87 to 200 μg/100 ml in the mother) However the enzyme deficiency was confirmed only in the mother whose OCT activity was equal to 13% of normal whereas the father had a normal OCT activity K_m values and the remaining urea cycle enzymes could not be measured notably carbamyl phosphate synthetase because of the small size of the biopsy specimens

Case 2

Vincent V is the youngest of 3 children His eldest sister now 4½ years is in good health (III Fig 2) One brother (III Fig 2) born in May 1966 died at age 5 months in a coma with seizures hypertonicity vomiting and psychomotor retardation The first symptoms appeared at 8 days of age with vomiting and lethargy suggestive of pyloric stenosis which was not found at operation Both parents are in good health and are not consanguineous The mother has 4 brothers and 4 sisters who have 24 healthy children with the exception of one female who died at 2 days of age of a congenital cardiac malformation A fifth brother was still born with a cerebral malformation (II Fig 2) The father has 2 sisters in good health one of which (II_{3}) has a mentally retarded daughter with a cardiac malformation and 2 brothers who were stillborn following traumatic deliveries

History of the proband

Vincent was born Oct 10 1972 at term by caesarian section due to cephalo-pelvic disproportion Symptoms appeared on the sixth day of life with severe vomiting abdominal distension tachypnea at 90/min Gasping moderate icterus hypertonicity of the extremities motivated his

Table 2 *Liver urea cycle enzyme activity expressed as μmoles of product released per m protein and per hour family no 1*

Controls 5 girls 6 boys (10 open or needle biopsies 1 post mortem biopsy)

Enzymes	Father	Mother	Patient	Controls ($n=11$)
Carbamyl phosphate synthetase			0 22	0 7±0 20
Ornithine transcarbamylase	34 3	4 1	2 82	32±5 8
Argininosuccinase			1 74	1±0 34
Arginase			96	77±20

months on February 15 1972 with anorexia asthenia and mild icterus Three days later he suffered from uncontrolled vomiting followed by diffuse abdominal pain which at first was suspected of being acute appendicitis A few hours later hospitalization was decided because of severe neurological signs chewing athetoid movements of the extremities somnolence interspersed with periods of agitation The child could no longer recognize his surroundings By the following day he was in a deep coma and presented a diffuse pyramidal syndrome with respiratory congestion The ocular fundi and the cerebrospinal fluid were normal

The child was transferred to an intensive care unit Laboratory findings on admission were RBC 4 2×10⁶/μl WBC 1 9×10⁴/μl with 82% PMN Plts 4 5×10⁵/μl pH *7 62 P_{CO_2} 19 mmHg BE +1 mEq/l blood sugar 206 mg/* 100 ml BUN 30 mg/100 ml Ca 10 2 mg/100 ml Na 144 mEq/l K 3 8 mEq/l Cl 106 mEq/l proteins 75 g/l Acetest (+) Bilirubin 0 5 mg/100 ml prothrombin 15% Factors VII+X 76% Factor V 80% Fibrinogen 1 8 g/l total cholesterol 150 mg/100 ml lactic acid 30 mg/100 ml The serum ammonia level was markedly elevated 1 370 μg/*100 ml and a few hours later 1 800 μg/100 ml* (measure by the diffusion method of Seligson normal <150 μ 100 ml) (19) Three hours after admission because c seizures the patient was treated with 40 mg of Valium (intravenously) and 80 mg of Pentothal® (per rectum) Th EEG showed a modified trace absence of basic rhythm with paroxysmal discharges alternating from side to sid One hour later ventricular tachycardia at 240/min ap peared the child was placed in a ventilator in addition peritoneal dialysis was undertaken in order to decreas the ammonia level At the end of this dialysis the bloo ammonia level fell to 145 μg/100 ml Nevertheless th course remained unfavorable both clinically and electro encephalographically with irreversible coma and deat *24 hours later on 21 February 1972*

Biochemical studies

Chromatography of the plasma amino acids (Technico Autoanalyzer) showed an increase in lysine (3 times nor mal) α alanine and glutamine There was an absence o citrulline and argininosuccinic acid Urinary chroma

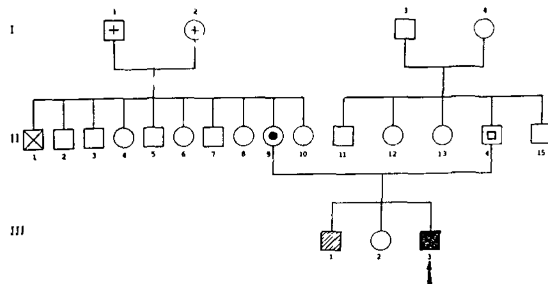


Fig 2 Pedigree of family 2

Stillborn

Unexplained death

Clinical symptoms of hyperammonemia with proved OCT deficiency

OCT deficiency without clinical symptoms

Normal OCT activity

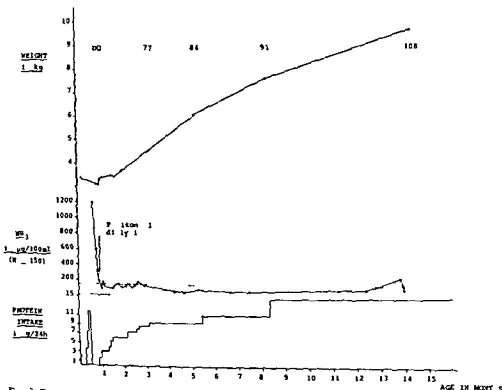


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The entire liver was removed immediately following death and frozen at −80°C The urea cycle enzymes were studied as previously described (6 7 8) There existed a severe OCT deficiency as measured under standard conditions (residual activity equal to 10% of normal) associated with a definite but less marked carbamyl phosphate synthetase deficiency (approximately 25% of normal) Argino succinase and arginase activities were not decreased (Table) There was no modification of the K of OCT for one or th other substrate K_m carbamyl phosphate 0 31×10⁻³ M (control 0 40–0 70×10⁻ M) K ornithine 0 44×10⁻³ M (control 0 35–0 5?×10⁻ M)

Study of the parents

Both mother and father were subjected to a protein loading test as well as needle biopsy of the liver for enzymatic studies The protein loading test (ingestion of 2 g of protein/kg) showed that both the father and the mother have a definite increase in their ammonia level at 4 hours following the meal (increase from 100 at 700 µg/100 ml in the father and from 87 to 700 µg/100 ml in the mother) However the enzyme deficiency was confirmed only in the mother whose OCT activity was equal to 13% of normal whereas the father had a normal OCT activity K_m values and the remaining urea cycle enzymes could not be measured notably carbamyl phosphate synthetase because of the small size of the biopsy specimens

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Table 3 *Plasma amino acids in µmoles/100 ml before and after peritoneal dialysis patien number 2*

	22 10 72	26 10 72 post dialysis	20 11 72	02 01 73	Controls
Taurine	16 9	14	18 2	2 5	7 4–21 6
Aspartic acid	6 6	3 5	0 9	1 2	0 5–?
Threonine	7 1	3 6	13 6	10 6	11 4–33 5
Serine	13 4	8 5	23	15 1	12 2–24 2
Glutamic acid			2 2	1 2	2–10 7
Proline	6	9 8	33 1	32	10–27
Glycine	19 2	22 5	41 8	27 2	22 4–51 4
α NH₂ butyr acid	2 7	0 8	0 8		0 8–4 9
Valine	7 7	6	13 6	12 7	8–24 6
1/2 Cystine	Traces	0 5	9 1	4 4	2–14
Methionine	1 6	0 5	0 9	3 3	0 9–4 1
Isoleucine	3 3	1 8	8 3	5 3	2 7–9 4
Leucine	6 1	4 7	9 1	8 2	4 7–10 9
Tyrosine	5 6	2	5 2	4 7	4 2–9 9
Phenylalanine	5 6	3 5	6 2	5 4	4 2–11
Ornithine	4 9	4	7 3	9 1	4 9–15 1
Lysine	41 3	7	14 3	13 4	11 4–25 9
Histidine	9 3	5 1	10 8	5 8	4 9–11 4
Arginine	3 8	3 8	3 5	2 9	2 2–8 8
α alanine	23 8	18 5	67 6	56 1	23 6–41
Glutamine	121	72			15–89
α NH₂ adipic acid	8 8	Traces	0	0	0

hospitalization in an intensive care unit On admission abdominal and chest X rays cerebrospinal fluid ocular fundi and electroencephalogram were normal Laboratory findings on admission were pH 7 52 P_{CO_2} 24 mmHg BE 0 mEq/l Proteins 57 g/l Blood sugar 175 mg/100 ml Ca 9 7 mg/100 ml Na 138 mEq/l Cl 110 mEq/l direct bilirubin 2 mg/100 ml transaminase SGOT 45 U Frankel SGPT 31 U Frankel (normal <40) Lactic acid 16 mg/100 ml The serum ammonia level was markedly elevated 1 200 µg/100 ml (normal <150) He was placed on a protein free diet and given 5% glucose intravenously The tachypnea diminished progressively and the icterus decreased however the child became hypotonic and the primary reflexes disappeared

At 15 days of age the child was transferred to the Hopital des Enfants Malades in Paris On admission his weight was 3 400 g He was well hydrated The neurological ex amination was striking following stimulation the chil opened his eyes widely in a fixed stare focused in th distance with rolling lateral movements He presente excesses of body extension with slow elevation of one o several extremities alternating with severe hypotonicity The deep tendon reflexes were present in the upper ex tremities and absent in the lower extremities There was a suggestion of the grasp reflex when awake negative Mor reflex weak sucking reflex The EEG showed diffus slowing with numerous abnormalities of the right hemi sphere

Acid–base balance blood glucose serum electrolytes blood cell counts were all normal The blood ammonia leve was 275 µg/100 ml (normal <150) in spite of the fact tha the child had not received any protein for 9 days Despit

Table 4 *Liver urea cycle enzyme activity expressed as µmoles of product released per mg protein and per hour family number 2*

Controls 5 girls 6 boys (10 open or needle biopsies 1 post mortem biopsy)

Enzymes	Father	Mother	Patient	Controls (n=11)
Carbamyl phosphate synthetase			0 70	0 7±0 20
Ornithine trans carbamylase	24 (K_m 0 87×10⁻³ M)	14 8 (K_m 0 31×10⁻³ M)	2 (K_m 2 4×10⁻³ M)	32±5 8 (K_m 0 35–0 52×10⁻³ M)
Argininosuccinase			1 33	1±0 34
Arginase			108	77±20

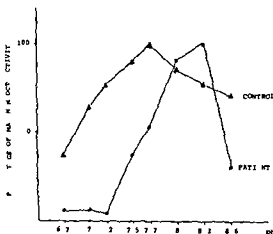


Fig 4 Effect of pH on the OCT activity from liver extract of patient no ? and control

the slight elevation in the ammonia level the clinical state remained serious and peritoneal dialysis was undertaken for 24 hours during which the patient was breathing spontaneously After 12 hours of dialysis a definite improvement was noted in his neurological status he was awake and more reactive the truncal spasms and abnormal movement of the extremities had disappeared primary reflexes returned General hypotonicity persisted however and the serum ammonia was 00 μg/100 ml Oral feeding began 24 hours following the end of dialysis with a minimal protein intake which was then progressively increased (Fig 3) Liver biopsy under general anesthesia was performed on 13 December 1972 without complications

Weight gain appeared towards the end of November and persisted allowing an increase in protein intake (Fig 3) At the same time the neurological status continued to improve as well as the development quotient as measured by the test of Brunet Lezine The electroencephalogram was normal The 2 hour postprandial ammonia levels were consistently below 150 μg/100 ml with the exception of one value at 195 μg/100 ml (Fig 3) The BUN remained low between 10 and 15 mg/100 ml and the urinary urea excretion was less than 1 g/24 h In December 1973 at 14 months of age the weight of the child was 9 6 kg and he was 7? cm tall His protein intake was 15 g/24 h given in 4 meals There was a transient episode of hyperammonianemia of 240 μg/100 ml during the course of an upper respiratory infection The development quotient according to the Brunet Lezine test was 108 The neurological examination was normal

Biochemical studies

The results of chromatography of the plasma amino acids (Technicon Autoanalyzer) are given in Table 3 The first chromatography on 22 Oct 197? at the age of 1? days showed a rise in the aspartic acid lysine and glutamine as well as a sharp peak in the α-amino adipic acid These abnormalities disappeared during the course of dialysis On 20 November 1972 a moderate elevation of α-alanine appeared The remainder of the chromatogram was normal Orotic acid in the urine (method of Rogers & Porter) has been consistently negative

Enzymatic assays

The assays were done on a biopsy specimen of the liver performed under general anesthesia and frozen immediately to −80 C An OCT deficiency existed as measured under standard conditions The residual enzymatic activity was approximately 6% of normal Carbamyl phosphate synthetase argino succinase and arginase activities were found to be normal (Table 4) The

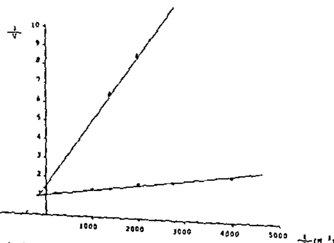


Fig 5 Lineweaver Burk plot of the OCT activity at increasing ornithine concentrations in presence of 5 mM carbamyl phosphate at optimum pH *Patient no 2*

K_m=2 4×10⁻³ M at pH 8 3 Control K_m=0 35×10⁻³ M at pH 7 7

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erine	13 4	8 5	23	15 1	12 2–24 2
ilutamic acid			2 2	1 2	2–10 7
'roline	6	9 8	33 1	32	10–77
ilycine	19 2	22 5	41 8	27 2	22 4–51 4
NH_2 butyr acid	2 7	0 8	0 8		0 8–4 9
'aline	7 7	6	13 6	12 7	8–24 6
/2 Cystine	Traces	0 5	9 1	4 4	2–14
lethionine	1 6	0 5	0 9	3 3	0 9–4 1
soleucine	3 3	1 8	8 3	5 3	2 7–9 4
.eucine	6 1	4 7	9 1	8 2	4 7–10 9
'yrosine	5 6	2	5 2	4 7	4 2–9 9
'henylalanine	5 6	3 5	6 2	5 4	4 2–11
)rnithine	4 9	4	7 3	9 1	4 9–15 1
.ysine	41 3	7	14 3	13 4	11 4–25 9
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Argininosuccinase			1 33	1±0 34
Arginase			108	77±20

mother had 50% OCT activity as compared with the normal controls In her case we did not find any abnormality in the affinity of the enzyme for either substrate (Table 4)

DISCUSSION

Until 1969 OCT deficiency had only been reported in families with female cases (with one exception the male case of Levin which was extremely mild (13)) Recently there have been a number of reports of observations in boys so that of a total of about 20 known families 11 deal with male cases (Table 5)

Both cases described here demonstrate well as opposed to what had been postulated by Short et al (21 22 23) that the forms of the disease seen in boys are not necessarily neonatal and fatal and especially that they do not always imply an almost total lack of OCT The second case in fact began in the neonatal period though following a free interval of one week which is significantly longer than the 24–48 hour delay found in other neonatal observations Moreover the outcome was favorable and the OCT deficiency was only partial with a persistent residual activity of 6% The first case proved to be fatal but became apparent quite late at 8 years of age and the *partial deficiency (10%) was compatible* with a near normal life for a prolonged period of time

Both these cases though not strictly neonatal and having a certain residual *in vitro* OCT activity are not unique in the literature and should be compared to the case of Levin *(13) cited above who had 25% residual activity* at pH 7 and to the male case of MacLeod who did not die until the fourth month with a residual OCT activity of 5% (14) In these 4 male cases the disease is compatible with life and is probably different from the 7 lethal observations in the newborn in which the enzymatic activity is consistently absent of less than 0 5%

However the X linked mode of transmission of OCT deficiency cannot be questioned by these 4 observations although in this hypo*thesis one would expect to find no discernible* activity in male hemizygotes In fact we have previously shown (5) that mutations affecting the OCT structural gene are heterogeneous in nature

With regard to the deficiency in males qualitative assays of OCT were performed in 5 cases (cases 1 2 7 10 11 Table 5) Three cases proved to have an abnormal K_m for one or the other of the OCT substrates permitting the identification of at least 3 variants of the disease

Even in the severe neonatal forms having an enzyme deficiency of less than 0 5% of normal the mutations are probably not homogeneous since the two cases in the family described by Campbell & Short (No 2 Table 5) (23) bore no demonstrable qualitative abnormality whereas our case No 7 (Table 5) had an elevated K_m for ornithine and an abnormal activity at alkaline pH (8 18)

These 3 male variants are related to a mutation of the structural gene of OCT and should be added to the female variants previously described in the literature a variant in which activity decreased abruptly at pH 5 (12) and two variants in which the K_m for carbamylphosphate was increased (15) and (7) *Since the two latter mutations are probably* identical it is likely that preferential mutation sites do exist Mutation can therefore more or less profoundly alter the enzymatic activity

In our 2 cases and those of Levin and MacLeod the enzyme is only slightly altered by the mutation The male hemizygotes are viable The female heterozygotes have variable enzymatic deficiencies which may be explained by *the lyon hypothesis which states that all* females are a mosaic of two populations of cells in which one or the other of their X chromosomes is inactivated (23)

The fact that the mother of case number 2 did not display the K_m abnormality which was present in her son may be accounted for by her having 50% residual activity due to her normal X chromosome such a high residual activity probably makes it impossible to detect in her case a slight variation in the K_m

Table 5 *Families with male cases reported in literature*

Authors	Patients				Family history
	Onset	OCT (%)	Qualitative abnormality	Course	
(1) Levin (1969) (13)	8–9 months	25% at pH 7 Normal at pH 8	Decreased K_m for ornithine and carbamyl phosphate	Normal at 2 years	Only child Normal protein load in the mother
(2) Campbell (1971 1973) and Short (1973) (1 2 3 23)	3 days	0 4%	Normal optimum pH	Death at 4 days	1 brother died at 4 days Normal protein load in the mother Normal maternal liver OCT
	36 hours	0 1%	Normal K_m for ornithine and carbamyl phosphate	Death at 9 days	
(3) Short (1972 1973) (pedigree C) (21 22 23)	48 hours	0		Death at 48 hours	4 male neonatal deaths in 2 generations OCT deficiency in one sister (23%) Abnormal protein load in the mother Maternal liver OCT 39% OCT deficiency in a maternal aunt 63%
(4) Scott (1972) (20)	48 hours	0		Death at 48 hours	Neonatal deaths 1 brother 1 maternal first cousin and 3 maternal uncles
(5) Scott (1972) (20)	Neonatal	0		Neonatal death	8 male neonatal deaths in 3 generations
(6) MacLeod (1972) (14)	5 weeks	5%		Death at 4 months	
(7) Saudubray & Cathelineau (1973) (18 8)	36 hours	0 2%	Increased K_m for ornithine activity at increased alkaline pH	Death at 11 days	5 male neonatal deaths in 2 generations
(8) Kang (1973) (11)	24 hours	0		Death at 5 days	Normal protein load in both parents
	24 hours	0 2%		Death at 5 days	
(9) Goldstein (1974) (10)	6 days	0 2%		Death at 25 days	2 brothers died at 7 days Oroticuria following protein load mother maternal grandmother and aunt
(10) Saudubray & Cathelineau Case II	6 days	6 5%	Increased K_m for ornithine Optimum pH shift toward alkaline side	Normal at 15 mos	1 brother died at 5 mos following seizures and psychomotor retardation Maternal liver OCT deficiency 50%
(11) Saudubray & Cathleineau Case I	8 years	10% associated with CPS deficiency (25%)	Normal K_m for ornithine and carbamyl phosphate	Death at 8 years	1 maternal uncle died at 2 years encephalopathy Maternal liver OCT deficiency 13%

optimum enzyme activity shifted towards the alkaline side (optimum activity at pH 8 3) whereas optimum activity in the control group was at 7 7 (Fig 4) In addition there was a significant decrease in the affinity of OCT for its substrate ornithine In fact at the optimum pH 8 3 the K_m value was increased $K_m = 2\ 4 \times 10^{-3}$ M whereas the K_m of the control at optimum pH was equal to $0\ 35 \times 10^{-3}$ M (Fig 5) The K_m for the substrate carbamyl phosphate was normal ($0\ 80 \times 10^{-3}$ M at optimum pH whereas the K_m of the control was $0\ 55 \times 10^{-3}$ M)

Study of the parents

Both parents underwent needle biopsy of the liver The father had an OCT activity within normal range The

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LACK OF RELATIONSHIP OF RED CELL ENZYME ACTIVITY TO BILIRUBIN AND CARBOXYHEMOGLOBIN LEVELS IN HEALTHY TERM INFANTS

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ABSTRACT Komazawa M Landaw S A and Oski F A (Departments of Pediatrics and Medicine State University of New York Upstate Medical Center Syracuse New York USA) Lack of relationship of red cell enzyme activity to bilirubin and carboxyhemoglobin levels in health term infants Acta Paediatr Scand 64 473 1975 —A total of 32 term infants were studied in an attempt to confirm and extend the recent observation of Petrich & associates (14) that minor degrees of transient deficiencies of the red cell enzymes glucose phosphate isomerase and glyceraldehyde 3 phosphate dehydrogenase were related to hyperbilirubinemia in other wise healthy term infants No relationships could be observed between the activity of these enzymes and the bilirubin level on day three In addition no correlation was present between bilirubin values and carboxyhemoglobin levels on day three suggesting that in the healthy term infant excessive hemolysis is not usually responsible for the variations observed in bilirubin levels

KEY WORDS Erythrocytes jaundice newborn bilirubin carboxyhemoglobin

The etiology of transient hyperbilirubinemia in the newborn infant remains unknown Presumably it is multifactoral in origin Accelerated rates of red cell destruction coupled with impaired bilirubin conjugation and increased entero hepatic recirculation of bilirubin may all contribute to idiopathic hyperbilirubinemia

Recently Petrich and associates (14) reported a correlation between the degree of bilirubin elevation in otherwise healthy term infants and the degree of inhibition of the activities of the red cell enzymes glucose phosphate isomerase (GPI) and glyceraldehyde 3 phosphate dehydrogenase (G 3 PD) The authors speculated that some minor degree of red cell enzyme deficiency might produce an increased rate of red cell destruction and thus be responsible for the hyper bilirubinemia

Because of the potential importance of these observations a study was undertaken in an attempt to confirm and extend these findings

MATERIALS AND METHODS

Patients

Thirty two term infants (birth weights 2 640–4 090 g) were studied on the third day of life All infants were apparently healthy and free of blood group incompatibilities or other recognized hematologic causes of hyperbilirubinemia

Blood was collected from heel stick punctures into heparinized capillary tubes

Methods

Whole blood was utilized for the measurement of hematocrit reticulocyte counts and the determination of carboxyhemoglobin and red cell 2 3-diphosphoglycerate levels

Plasma was employed for the measurement of bilirubin Erythrocytes were utilized for the measurement of hexokinase glucose phosphate isomerase phosphofructo-

REFERENCES

1 Campbell A G M Rosenberg L E Snodgrass P J & Nuzum C T Lethal neonatal hyperammonemia due to complete ornithine transcarbamylase deficiency *Lancet II* 217 1971

2 — Complete ornithine transcarbamylase deficiency a cause of lethal neonatal hyperammonemia *Pediatr Res 5* 384 1971

3 — Ornithine transcarbamylase deficiency A cause of lethal neonatal hyperammonemia in Males *N Engl J Med 288* 1 1973

4 Cathelineau L Navarro J Polonovski C & Saudubray J M X linked transmission of structural gene mutations responsible for ornithine trans carbamylase (OCT) deficiencies *Lancet I* 261 1973

5 Cathelineau L Saudubray J M Navarro J & Polonovski C Transmission par le chromosome X du gene de structure de l ornithine carbamyl transférase Etude de trois familles *Ann Genet 16* 173 1973

6 Cathelineau L Saudubray J M & Polonovski C Mutations on human ornithine carbamyltransferase *Enzyme 18* 103 1974

7 *Cathelineau L Navarro J Aymard P Baudon* J J Mondet Y Polonovski C & Laplane R Hyperammoniemie héréditaire par anomalie qualitative de l ornithine carbamyl transférase hépatique et intestinale *Arch Fr Pediatr 29* 713 1972

8 Cathelineau L Saudubray J M & Polonovski C Ornithine carbamyl transferase the effects of pH on the kinetics of a mutant human enzyme *Clin Chim Acta 41* 305 1972

9 Giorgio A J & Plaut G W E A method for the colorimetric determination of urinary methylmalonic acid in pernicious anemia *J Lab Clin Med 66* 667 1965

10 Goldstein A S Hoogenraad N J Johnson J D Fukanaga K Swierczewski E Cann H M & Sunshine P Metabolic and genetic studies of a family with ornithine transcarbamylase deficiency *Pediatr Res 8* 5 1974

11 Kang E S Snodgrass P J & Gerald P S Ornithine transcarbamylase deficiency in the newborn infant *J Pediatr 8?* 642 1973

12 Levin B Abraham J M Oberholzer V G & Burgess E A Hyperammonaemia a deficiency of liver ornithine transcarbamylase Occurrence in mother and child *Arch Dis Child 44* 152 1969

13 Levin B Dobbs R H Burgess E A & Palmer T Hyperammonaemia A variant type of deficiency of liver ornithine transcarbamylase *Arch Dis Child 44* 162 1969

14 MacLeod P Mackenzie S & Scriver C R Partial ornithine carbamyl transferase deficiency an inborn error of the urea cycle presenting as orotic aciduria in a male infant *Can Med Assoc J 107* 405 1972

15 *Matsuda I Arashima S Nambu H Takekoshi* Y Anakura M Hyperammonemia due to a mutant enzyme of ornithine transcarbamylase *Pediatrics 48* 595 1971

16 Rogers L E & Porter F St Hereditary orotic aciduria II A urinary screening test *Pediatrics 4? 423* 1968

17 Russell A Levin B Oberholzer V G & Sinclair L Hyperammonemia a new instance of an inborn *enzymatic defect of the biosynthesis of urea Lancet II* 699 1962

18 Saudubray J M Cathelineau L Charpentier C *Boisse J Allaneau C Le Bont H & Lesage B* Deficit héréditaire en ornithine carbamyl transferase avec anomalie enzymatique qualitative Relation d une forme à *révélation néonatale et d evolution* rapidement mortelle chez un garçon *Arch Fr Pediatr 30* 15 1973

19 *Seligson D & Hirahara K The measurement of* ammonia in whole blood erythrocytes and plasma *J Lab Clin Med 49* 96? 1957

20 Scott C R Teng C C *Goodman S I Greensher* A & Mace J W X linked transmission of ornithine transcarbamylase deficiency *Lancet II* 1148 1972

21 Short E M *Conn H O Snodgrass* P J & Rosenberg L E Evidence of X linked inheritance of ornithine transcarbamylase deficiency *J Clin Invest 24* 90a *1972*

22 — X linked dominant inheritance of ornithine trans carbamylase deficiency *Am J Hum Genet 24* 24a 1972

23 Short E M Conn H O Snodgrass P J Campbell A G M & Rosenberg L E Evidence for X linked *dominant inheritance of ornithine* transcarbamylase deficiency *N Engl J Med 288* 7 1973

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level and the percentage of carboxyhemoglobin This finding indicates that the hyperbilirubinemia observed in healthy term infants is not primarily the result of increased red cell breakdown and is consistent with the failure to find any relationship between parameters of red cell metabolism and bilirubin level

DISCUSSION

Our studies have failed to confirm the observations of Petrich and associates (14) that mild enzyme deficiencies principally of glucose phosphate isomerase and glyceraldehyde 3 phosphate dehydrogenase were responsible for the hyperbilirubinemia of term infants We were also unable to demonstrate an inverse relationship between bilirubin level and red cell 2 3 DPG concentration as reported by these authors

Elevations in bilirubin levels alone cannot be used as proof that excessive hemolysis is present in the newborn infant The rise in bilirubin may be caused by impaired conjugation of bilirubin or excessive entero hepatic circulation of bilirubin The measurement of carboxyhemoglobin in conjunction with the measurement of bilirubin is far more sensitive index of the presence of hemolysis (4 7) The destruction of red cells gives rise to both one mole of bilirubin and one mole of carbon monoxide In clinical conditions in which hyperbilirubinemia is a result of increased red cell destruction carbon monoxide production rates are increased and blood carboxyhemoglobin values are elevated Carboxyhemoglobin values obtained on the first day of life are frequently difficult to interpret because of maternal contributions to newborn levels as a result of smoking or atmospheric exposure Carboxyhemoglobin values on day three of life accurately reflect the infant s endogenous carbon monoxide production when potential nursery pollution has been eliminated as a cause

The lack of correlation between bilirubin levels and carboxyhemoglobin values observed in this study suggests that the elevations in bilirubin were not a result of primary alterations in red cell metabolism The lack of relationship between hematocrit values reticulocyte counts and bilirubin levels also support this conclusion

Minimal decrease in enzyme activity as reported by Petrich and co workers would not be anticipated to produce alterations in red cell metabolism In most instances individuals who are heterozygous for enzyme deficiencies such as pyruvate kinase deficiency hexokinase deficiency and glucose phosphate isomerase deficiency have no evidence of a hemolytic process despite the fact that enzyme activity is reduced to 50% of normal (9) Red cell phosphofructokinase activity was included in this study in hopes of finding a relationship between this enzyme and hyperbilirubinemia because of all the glycolytic enzymes in the newborn s erythrocytes this enzyme is most consistently lower in value by adult standards (3 8 13) No relationship was found to exist between relative deficiencies of phosphofructokinase and bilirubin values

Landaw et al (10) previously examined the relationship between carboxyhemoglobin levels and bilirubin in healthy term infants and concluded that excessive hemolysis was not responsible for the bilirubin elevations Our studies also indicate that in the healthy term infant excessive hemolysis is not usually responsible for transient hyperbilirubinemia

REFERENCES

1 Alden E R Lynch S R & Wennberg R P Carboxyhemoglobin determination in evaluating neon tal jaundice Am J Dis Child 127 214 1974
2 Beutler E Red Cell Metabolism A Manual of Biochemical Methods Grune & Stratton Inc New York 1971
3 Caruso P Conti F & Londrillo A Diagramma delle attività enzimaticke endoentrocitarie nel neonato nel lattante nel bambino Minerva Pediatr 15 1136 1963
4 Coburn R F Endogenous carbon monoxide production N Engl J Med 282 207 1970

Table 1 *Results of hematologic studies*[a]

		N
Bilirubin (mg/100 ml)	7 70±3 67	32
Hct (%)	54 6±5 97	32
DPG (μMoles/ml RBCs)	6 27±1 09	31
Hexokinase (units/100 ml RBCs)	0 92±0 164	32
Glucose phosphate isomerase (units/100 ml RBCs)	24 03±4 253	32
Phosphofructokinase (units/100 ml RBCs)	3 125±0 875	32
Glyceraldehyde 3 phosphate dehydrogenase (units/100 ml RBCs)	105 09±18 24	28
Reticulocyte (%)	1 756±0 909	32
Carboxyhemoglobin (%)	0 5560±0 1033	25

[a] Mean±1 S D

kinase and glyceraldehyde 3 phosphate dehydrogenase activity

Specimens collected for carboxyhemoglobin determinations were sealed until analysis and stored at 4°C Carboxyhemoglobin was determined by the gas chromatographic technique of Collison et al (5) Simultaneous collections of room air in the nursery were obtained at the time of blood sampling After analysis of room air the contribution of exogenous carbon monoxide was subtracted from the blood level

Measurement of red cell 2 3-diphosphoglycerate levels were performed by the assay technique of Schroter & Heyden as previously described (6) Total bilirubin was measured by the technique of White et al (16) Activities of red cell hexokinase glucose phosphate isomerase and phosphofructokinase were determined by previously published methods (13) while the activity of glyceraldehyde 3 phosphate dehydrogenase was measured by the procedure described by Beutler employing non destromatized hemolysates prepared by hypotonic lysis (2) A substrate concentration of 5 mM *d*-glyceraldehyde 3 phosphate was utilized and the first 20 seconds of the reaction was measured for purposes of calculation of enzyme activity

RESULTS

In Table 1 the mean and standard deviation for each of the measurements performed is presented For technical reasons only 25 specimens were available for carboxyhemoglobin determinations 28 for G 3 PD assays and 31 for red cell 2,3 DPG determinations

Enzyme values for HK GPI and PFK are similar to those previously reported (3) Values for G 3 PD are considerably higher Our previous study employed destromatized hemolysates The enzyme G 3 PD is largely stroma bound (15) and the change in preparation of th hemolysates accounts for the increased activit observed

Values for bilirubin (11) carboxyhemoglobi (1) and red cell 2 3 DPG are in good agreemen with previously established normal values fo term infants (6 12)

In Table 2 are presented the correlation co efficients among combinations of all parame ters measured It will be noted that no signifi cant correlation exists among the activities o any of the enzymes measured and the bilirubin carboxyhemoglobin or 2 3 DPG concentra tions The only relationship that proved sig nificant was that between the activities o the enzymes GPI and G 3 PD The relation ships between GPI and hexokinase and G 3 PI and hexokinase approached statistical signifi cance suggesting that all these enzymes wer elevated

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Table 2 *An examination of the relationship between variables*

Bil=bilirubin PFK=phosphofructokinase GPI=glucose phosphate isomerase Hx=hexokinase GAPD=glycer aldehyde 3 phosphate dehydrogenase Hct=hematocrit CoHb=carboxyhemoglobin Ret=reticulocytes DPG= diphosphoglycerate

Vari able (1)	Vari able (2)	N	Corr Co ef	Slope	p
Bil	GAPD	28	0 1770	0 9166	>0 1
Bil	Hx	32	0 2002	0 0089	>0 1
Bil	GPI	32	0 2849	0 3310	>0 1
Bil	PFK	32	0 1262	0 0301	>0 1
Bil	DPG	31	0 1747	3 7226	>0 1
Bil	Ret	32	−0 2133	−0 0529	>0 1
Bil	CoHb	25	0 2564	0 0071	>0 1
PFK	GAPD	28	−0 0417	0 8204	>0 1
PFK	Hx	32	−0 0177	−0 0033	>0 1
PFK	GPI	32	0 0031	0 0153	>0 1
GPI	Hx	32	0 3152	0 0121	>0 05
GPI	GAPD	28	0 7317	3 0794	<0 001
Hx	GAPD	28	0 3082	33 5452	>0 05
GAPD	DPG	27	0 0087	0 0546	>0 1
Hct	DPG	31	−0 1035	−1 9128	>0 1
CoHb	GAPD	23	0 0538	16 9935	>0 1
CoHb	Hx	25	0 2386	0 3631	>0 1
CoHb	GPI	25	0 3707	14 3339	>0 05
CoHb	PFK	25	0 1640	1 4189	>0 1
CoHb	DPG	24	0 0147	16 3395	>0 1
Ret	CoHb	25	−0 3463	−0 0359	

level and the percentage of carboxyhemoglobin This finding indicates that the hyperbilirubinemia observed in healthy term infants is not primarily the result of increased red cell breakdown and is consistent with the failure to find any relationship between parameters of red cell metabolism and bilirubin level

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1 Alden E R Lynch S R & Wennberg R P Carboxyhemoglobin determination in evaluating neonatal jaundice *Am J Dis Child* 127 214 1974
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4 Coburn R F Endogenous carbon monoxide production *N Engl J Med* 282 207 1970

5 Collison H A Rodkey F L & O Neal J D Determination of carbon monoxide in blood by gas chromatography *Clin Chem* *14* 162 1968
6 Delivoria Papadopoulos M Roncević N P & Oski F A Postnatal changes in oxygen transport of term premature and sick infants The role of functioning fraction of red cell 2 3-diphosphoglycerate *Pediatr Res* *5* 235 1971
7 Fallstrom S P On the endogenous formation of carbon monoxide in full term newborn infants *Acta Paediatr Scand* Suppl *189* 1–27 1969
8 Gross R T Schroeder E A R & Brounstein S A Energy metabolism in the erythrocytes of the premature infant compared to full term newborn infants and adults *Blood* *21* 755 1963
9 Jaffe E R Clinical profile Hereditary hemolytic disorders and enzymatic deficiencies of human erythrocytes *Blood* *35* 116 1970
10 Landaw S A Kandall S R & Thaler M M Corrected carboxyhemoglobin as an index of hemolysis in non hemolytic neonatal hyperbilirubinemia *Clin Research* *21* 321 1973
11 Odell G B Physiologic hyperbilirubinemia in the neonatal period *N Engl J Med* *277* 193 1967
12 Orzalesi M M & Hay W W The regulation of oxygen affinity of fetal blood *Pediatrics* *48* 857 1971
13 Oski F A Red cell metabolism in the newborn infant V Glycolytic intermediates and glycolytic enzymes *Pediatrics* *44* 76 1969
14 Petrich C H Gempp Friedrich W & Gobel V Comparative measurements of enzyme activities and 2 3-diphosphoglycerate in the erythrocytes of newborns with transitory hyperbilirubinemia *Acta Paediatr Scand* *62* 596 1973
15 Skin B C & Carraway K L Association of glyceraldehyde 3 phosphate dehydrogenase with the human erythrocyte membrane *J Biol Chem* *248* 1436 1973
16 White D Abu Haider G & Reinhold J G Spectrophotometric measurement of bilirubin concentrations in the serum of the newborn *Clin Chem* *4* 211 1958

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SCREENING FOR CYSTIC FIBROSIS BY ANALYSIS OF ALBUMIN IN MECONIUM

H KOLLBERG and K HELLSING

From the Departments of Paediatrics and Clinical Chemistry University Hospital Uppsala Sweden

ABSTRACT Kollberg H and Hellsing K (Departments of Paediatrics and Clinical Chemistry University Hospital Uppsala Sweden) Screening for cystic fibrosis by analysis of albumin in meconium Acta Paediatr Scand 64 477 1975 —A clinical study of the albumin content in meconium was performed on two categories of newborn infants a screening series of 8 830 infants and a high risk group for Cystic Fibrosis (CF) of 70 infants A single radial immunodiffusion technique and test strips were used Three CF infants were detected in the screening series (1 3 000) and 16 in the high risk group The diagnostic accuracy for CF was fairly good The specificity was 99 8 % for the immunodiffusion technique and 99 2 % for test strips A high concentration of albumin in meconium was found not only in CF but also in preterm babies and infants with gastrointestinal disturbances such as atresias malaena neonatorum and malabsorption syndromes The sensitivity was 90 % for the immunodiffusion technique and 78 % for the test strip False negative results were probably due to proteolytic activity and might be avoided if the samples are stored at a low temperature before analysis CF screening of all meconiums by the use of test strips followed by analysis of positive tests by the *immunodiffusion technique is suggested*

KEY WORDS Albumin Cystic Fibrosis Immunodiffusion technique meconium screening

Cystic Fibrosis (CF) a generalized hereditary disease with dysfunction of exocrine glands is one of the principal causes of chronic illness in children and adolescents and one of the main causes of death in childhood in the Western countries (4) Early diagnosis and treatment are the best ways of improving the outcome for patients with CF (6 19 24) There is therefore a pressing need for a reliable screening test which can be used in the newborn period

Many different methods have been suggested as screening tests for CF In recent reviews (1 3) analysis of albumin in meconium has been considered to be the most promising

It is well known that meconium from CF infants has a higher concentration of albumin than other meconiums (for references see 9) In 1969 we started developing an immunochemical method for quantitative analysis of albumin in meconium (9 14) At the same time Stephan et al have been working on a test strip for qualitative analysis (21)

The aim of this study was to evaluate the clinical feasibility of the two methods and thus to find a practicable way of screening for CF in mass health examinations of newborns

MATERIAL

Meconiums were collected from two categories of newborn infants

1 A screening series consisting of 8 830 consecutive infants born in the following hospitals and periods (*a*) 7 813 from Uppsala University Hospital (Sept 1 1971 to Dec 31 1973) and Enköping Hospital (May 1 1972 to Dec 31 1973) and (*b*) 1 017 from Gävle Central Hospital (March 1 1973 to Dec 31 1973)

2 70 newborn infants who were at high risk of having CF

5 Collison H A Rodkey F L & O Neal J D Determination of carbon monoxide in blood by gas chromatography *Clin Chem 14* 162 1968
6 Delivoria Papadopoulos M Roncević N P & Oski F A Postnatal changes in oxygen transport of term premature and sick infants The role of functioning fraction of red cell 2 3-diphosphoglycerate *Pediatr Res 5* 235 1971
7 Fallstrom S P On the endogenous formation of carbon monoxide in full term newborn infants *Acta Paediatr Scand* Suppl *189* 1–27 1969
8 Gross R T Schroeder E A R & Brounstein S A Energy metabolism in the erythrocytes of the premature infant compared to full term newborn infants and adults *Blood 21* 755 1963
9 Jaffe E R Clinical profile Hereditary hemolytic disorders and enzymatic deficiencies of human erythrocytes *Blood 35* 116 1970
10 Landaw S A Kandall S R & Thaler M M Corrected carboxyhemoglobin as an index of hemolysis in non hemolytic neonatal hyperbilirubinemia *Clin Research 21* 321 1973
11 Odell G B Physiologic hyperbilirubinemia in the neonatal period *N Engl J Med 277* 193 1967
12 Orzalesi M M & Hay W W The regulation of oxygen affinity of fetal blood *Pediatrics 48* 857 1971
13 Oski F A Red cell metabolism in the newborn infant V Glycolytic intermediates and glycolytic enzymes *Pediatrics 44* 76 1969
14 Petrich C H Gempp Friedrich W & Gobel V Comparative measurements of enzyme activities and 2 3 diphosphoglycerate in the erythrocytes of newborns with transitory hyperbilirubinemia *Acta Paediatr Scand 62* 596 1973
15 Skin B C & Carraway K L Association of glyceraldehyde 3 phosphate dehydrogenase with the human erythrocyte membrane *J Biol Chem 248* 1436 1973
16 White D Abu Haider G & Reinhold J G Spectrophotometric measurement of bilirubin concentrations in the serum of the newborn *Clin Chem 4* 211 1958

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SCREENING FOR CYSTIC FIBROSIS BY ANALYSIS OF ALBUMIN IN MECONIUM

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(1969–73) i e patients with meconium ileus sibs of CF children and newborns with *meconium plug syndrome or* unusually viscous meconium

METHODS

A specimen of meconium was collected in a plastic vial either at defecation or from the napkin If possible the first-delivered part of the meconium was collected At the beginning of the study the specimens were kept at room temperature for up to 24 hours and the specimens from other hospitals were sent to us by ordinary mail When we found that the concentration of albumin fell rapidly at room temperature but was more stable at +4 C (9) the collection routine was changed in May 1972 and thereafter the vials with meconium were immediately placed in a refrigerator at +4 C and were mailed either deep frozen or at +4 C

The method described earlier for analysis of albumin in meconium by a single radial immunodiffusion technique (9) was employed The only modification consisted of the use of small disposable scoops instead of weighing each meconium sample The mean dry weight of meconiums from 65 such scoops was 94 3 mg (S D 12 2) This value was checked by weighing 30 meconium samples out of every 1 000 analysed and no essential drift occurred In order to detect all infants with more than 5 mg albumin/g meconium all meconiums with more than 3 5 mg/g (i e 1 25% of the samples) in the modified procedure were weighed and reanalysed All the analyses by test strips (BM Test Mekonium Boehringer Mannheim BRD) (21) from Uppsala and Enkoping were done in the laboratory mainly by the same technical assistant

The meconium samples from Uppsala and Enkoping were analysed in Uppsala by the immunodiffusion technique and from June 1972 also by test strips All meconiums *which were positive with the test strip at Gavle Central* Hospital were sent to Uppsala for quantitative measurements of albumin by the immunodiffusion technique

In the screening series all 33 infants with more than 10 mg albumin/g meconium and 26 out of 35 with 5–10 mg/g were subjected to a sweat test by pilocarpine iontophoresis (2) The concentration of sodium in the sweat was determined by a flame photometric method and the chloride concentration by a mercuric nitrate titrimetric method

In evaluating the discriminating ability of the immunodiffusion technique one sided tolerance intervals were used giving an upper limit for the normal and a lower limit for the CF infants Since it was not appropriate to make any assumption of the distribution a non parametric method (13) was used

RESULTS

The albumin concentrations obtained by the single radial immunodiffusion technique are given in Table 1 A chi square test revealed a tendency to lower concentrations of albumin in meconiums stored at room temperature than in those stored at +4°C ($p<0.10$)

The discriminating ability of the immunodiffusion technique was assessed from the values of meconiums stored at +4°C in the Uppsala screening series and of 17 CF meconiums (2 CF meconiums which had been stored for 5 days at room temperature were excluded) This gave a *probability of 99 9% that 99 5% of meconiums* from non CF infants would have an albumin concentration below 20 mg/g dry weight and a probability of 75% that 90% of meconiums from CF infants would have a concentration above this value

Analyses with test strips were performed on 5 756 meconiums from the screening series (including 1 from a CF infant) and on 48 meconiums from the group of newborns at risk (including 8 from CF infants) From 9 CF meconiums 7 strip tests were positive and 2 negative Another 47 test strips were positive (45 from the screening series and 2 from infants in the high risk group)

The sensitivity i e the degree of *ability to* detect CF (23), was 89% for the immunodiffusion technique and 78 for the test strip method in the total material and 100% for both methods in the screening series The specificity *i e the degree of ability to screen out those* who do not have CF (23) was 99 8% for the immunodiffusion technique and 99 2 for the test strip method both in the total material and in the screening series

All 35 infants with more than 20 mg albumin per g dry weight meconium were thoroughly investigated Their diagnoses are summarized in Table 2 Besides the 17 CF infants most of the high values came from preterm and small *for date infants (13 and 3 respectively)* Eight of these also had gastrointestinal disturbances viz 3 melaena neonatorum 3 atresia of the bowel and 2 malabsorption Of the 2 infants with malabsorption one probably had a Zellweger s syndrome (16) with a pathologically low content of chymotrypsin in faeces The other had gastrointestinal problems during the first year of his life with vomitings *loose*

Table 1 *Albumin concentration obtained by single radial immunodiffusion technique*

Material	Albumin concentration (mg/g) −0 99	1 0–4 9	5 0–19	20–99	≥100	Totally
Screening Uppsala Enkoping (n=7 813)						
Room temp (No strip tests)	2 921	132	16	2+1 CF	1+1 CF	3 072+2 CF
Stored at +4 C						
(Neg strip tests)	4 425	253	25	1	1	4 705
(Pos strip tests)	10	8	9	4	3	34
Total	7 356	393	50	7+1 CF	5+1 CF	*7 811+2 CF*
Screening Gavle (n=1 017)						
Neg strip tests						1 005
Pos strip tests	5	3	1	2+1 CF		11+1 CF
Total						*1 016+1 CF*
Group at high risk of having CF (n=70)						
Sibs of CF children	12	2+1 CF		1 CF	4 CF	14+6 CF
Meconium ileus			1 CF	5 CF	4 CF	10 CF
Intestinal obstruction	6	4	1	1	2	14
Viscous meconium	17	6	2		1	25
Total	35	12+1 CF	3+1 CF	1+6 CF	3+8 CF	*54+16 CF*

watery stools and poor weight gain he improved on a gluten free diet but a jejunal biopsy could not confirm a diagnosis of coeliaci and he is now doing quite well without any diet or other prescriptions The remaining two infants were born at term with a normal birth weight and seemed perfectly healthy with no gastrointestinal problems and with normal sweat tests Thus no reason for the high albumin concentration in their meconiums could be found

Three infants with CF were found in the screening series of 8 830 infants which means an incidence of about 1 3 000 newborns

In the series of infants at risk 2 CF infants were suspected of having CF although they had less than 20 mg albumin/g meconium and negative test strips In these cases the values were 1 1 mg/g and 15 mg/g but both these samples had been sent to us by routine mail without any cold packaging and arrived after 5 days

DISCUSSION

Specificity

An increased amount of albumin in the meconium is not specific for CF All newborns with low proteolytic activity in the meconium or an increased loss of albumin to the intestinal tract may have high concentrations of albumin in the meconium and thus show positive results in albumin assays

The clinical conditions found in this study are summarized in Table 2

In CF it is not clear whether the increased amount of albumin is due to decreased proteolytic activity and originates from swallowed amniotic fluid (10) or to increased loss to the intestinal tract (17) or to both

An increased *protein concentration in the* meconiums of *preterm infants* has been demonstrated earlier (5)

High loss of albumin to the intestine occurs in *melaena neonatorum* and in *exudative enteropathies* Positive tests in these conditions might be due to a great loss even with a normal proteolytic activity or to a moderate loss combined with a low proteolytic activity such as in preterm babies Three infants in our series had the combination of low gestational age melaena neonatorum and high concentration of albumin in the meconium

Intestinal obstruction due to meconium ileus and CF always gave high concentrations of albumin in the meconium in our series Meconiums from other intestinal obstructions

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Table 2 *Diagnosis of all children with more than 20 mg albumin/g dry weight meconium*

Diagnosis	Screening populations *n*	Clinically suspected *n*	Total *n*	Concentrations (mg/g)
Cystic fibrosis	3	14	17	22 35 39 44 62 70 74 77 80 160 200 280 310 330 350 400 720
Preterm infants				
Without gastrointestinal disturbances	7	1		29 33 42 63 67 100 210 420
With gastrointestinal disturbances				
Melaena neonatorum			13	
Duodenal atresia	3			31 150 180
Jejunal atresia		1		25
		1	8	Up to 120
Full term small for date infants				
With gastrointestinal disturbances				
Anal atresia			3	
Malabsorption	2	1		52
Full term healthy infants	2			80 290
Total			2	20 42
	17	18	35	

—duodenal jejunal and anal—varied from very low to pathologically high concentrations

CF is the main aetiologic factor in low proteolytic activity with *malabsorption* during early infancy But there are also other rare syndromes with low proteolytic activity such as pancreatic insufficiency with neutropenia (20), congenital malformations of the pancreas (11) and enterokinase deficiency (8)

The fact that the test is not specific for CF is no drawback in itself since it is of great importance to be able to recognize early any clinical conditions with high concentrations of albumin in the meconium

Table 3 *Estimated cost of screening for CF in Sweden (Sw kr)*

Test strips (120 000)	367 000
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Total/year	406 000
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The costs are based on the following assumption Birth rate 120 000 infants per year Positive test strips (1 %) are analysed by the immunodiffusion technique Sweat tests are performed on infants with more than 20 mg albumin/g meconium (10 out of 46 see Table 1) CF incidence 1 3 000 and sensitivity of the test 90% All costs are included (wages supervision material apparatus laboratory postage administration and registration) Values are given in Swedish crowns 1 Sw kr = U S $0 22

Sensitivity

The figures for sensitivity given in our series are very uncertain They might be too low since we included 2 CF specimens which had been stored under uncontrolled temperature conditions for a long time and had low albumin concentrations It has been shown that the concentration of albumin in meconium falls rapidly at room temperature (9)

The figures might on the other hand be too high if some CF patients in the series have not yet been revealed The risk of having missed some of the CF infants seems however to be relatively small From several screening series with a total of 69 000 infants (22) only 2 additional CF infants with negative strip tests have been reported (12)

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Total/diagnosed CF (406 000/36)	11 300

The costs are based on the following assumption Birth rate 120 000 infants per year Positive test strips (1 %) are analysed by the immunodiffusion technique Sweat tests are performed on infants with more than 20 mg albumin/g meconium (10 out of 46 see Table 1) CF incidence 1 3 000 and sensitivity of the test 90% All costs are included (wages supervision material apparatus laboratory postage administration and registration) Values are given in Swedish crowns 1 Sw kr = U S $0 22

Sensitivity

The figures for sensitivity given in our series are very uncertain They might be too low since we included 2 CF specimens which had been stored under uncontrolled temperature conditions for a long time and had low albumin concentrations It has been shown that the concentration of albumin in meconium falls rapidly at room temperature (9)

The figures might on the other hand be too high if some CF patients in the series have not yet been revealed The risk of having missed some of the CF infants seems however to be relatively small From several screening series with a total of 69 000 infants (22) only 2 additional CF infants with negative strip tests have been reported (12)

The large differences in albumin concentrations between CF meconiums might indicate a difference in their proteolytic activity This

activity may be completely absent in those with the highest concentrations It is possible however that about 10% of CF infants may have proteolytic activity at birth (7) In some of these the test for CF may be negative while others still have an increased amount of albumin in the meconium and are detected by the screening test

When interpreting the results of albumin determinations in meconium both the time and temperature of storage have to be taken into consideration After some time at room temperature analyses of some CF meconiums by the test strips might be negative A moderate increase of the albumin concentration found by the quantitative method could then be the only way of suspecting CF

Screening

The study has shown that various conditions with a high albumin content in the meconium are revealed both by the immunodiffusion technique and by the test strip It is important that these clinical conditions are detected in the neonatal period In the screening series consisting of a truly random sample about 15% of the meconiums containing more than 20 mg albumin/g came from CF infants

If the incidence of CF is about 1 3 000 about 40 CF infants are born in Sweden per year This figure which was derived from the finding of 3 CF infants in 8 830 newborns is of course very uncertain—the 95% confidence limits are approximately 1 11 400–1 920

A test strip meets most of the requirements for a screening device (25) it is easy to handle and applicable to large series the samples are easily obtained without any harm to the patient and the cost of the method is acceptable The diagnostic accuracy for CF is fairly good The sensitivity will probably be 90% or more with correct handling of the specimens The specificity is however rather low and about 1% of meconiums would be positive Positive tests must lead to further investigations for CF If the meconiums with positive strip tests are analysed by the immunodiffusion technique at least half of them will be excluded from suspicion of CF If the rest of the infants undergo sweat tests nearly all CF infants will be revealed The total cost per discovered CF infant would be about 11 300 Sw kr (Table 3)

Thus we are convinced that screening for CF is meaningful and should be started

It is important however to remember that there probably always will be false negative results and it must be stressed that the diagnosis of CF is not excluded by a finding of a normal albumin concentration in the meconium

Wherever a screening investigation for CF is started there must be possibilities for confirmative diagnosis by pilocarpine iontophoresis and for adequate medical and psychological treatment (25) When large series of CF patients diagnosed at birth and treated according to well defined principles are reported we might get an answer as to the most suitable treatment (15)

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Gunnar Ekbohm M A has kindly helped us with the statistical analyses

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Case reports was kindly made from the Department of Paediatrics Boden Hospital and from the Department of Child Surgery St Goran Hospital Stockholm Sweden

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REFERENCES

1 Bender S W Conrad H Chr & Biener G Screening auf Mucoviscidose (Cystische Fibrose/CF) *Monatsschr Kinderheilkd 119* 632 1971

2 Berg T & Wranne L *A modification of the sweat test by the pilocarpine iontophoresis method* Report at the 15th Nordic Ped Congress Bergen 1967

3 Bray P T *Report from the screening committee* European Working Group for Cystic Fibrosis London 1971

4 di Sant Agnese P A & Talamo R C Pathogenesis and physiopathology of cystic fibrosis of the pancreas *N Engl J Med 277* 1287 1967

5 Eggermont E Enzymic activities in meconium from human foetuses and newborns *Biol Neonate 10* 266 1966

6 George L & Norman A P Life tables for cystic fibrosis *Arch Dis Child 46* 139 1971

7 Green M N & Shwachman H Presumptive tests for cystic fibrosis based on serum protein in meconium *Pediatrics 41* 989 1968

8 Hadorn B Tarlow M J Lloyd J K & Wolff O H Intestinal enterokinase deficiency *Lancet I* 812 1969

9 Hellsing K & Kollberg H Analysis of albumin in meconium for early detection of cystic fibrosis A methodological study *Scand J Clin Lab Invest 33* 333 1974

10 Hobbs J R First meconium in the detection of fibrocystic disease of the pancreas *Protides of the Biological Fluids 17* 517 1969

11 Hurwitt E S & Arnheim E E Meconium ileus associated with stenosis of the pancreatic duct *Am J Dis Child 64* 443 1942

12 Kaiser D Manotti M & Remert O M Mukovis zidose bei negativem BM Mekoniumtest To be published

13 Kollberg H & Ekbohm G A clinical study of the diagnosis of cystic fibrosis by instrumental neutron activation analysis of sodium in nail clippings *Acta Paediatr Scand 63* 411 1974

14 Kollberg H & Hellsing K Screening for cystic fibrosis *Arch Dis Child 47* 836 1972

15 Lawson D Cystic fibrosis—Assessing the effects of treatment *Arch Dis Child 47* 1 1972

16 Opitz J M ZuRhein G M Vitale L Shahidi N T Howe J J Chou S M Shanklin D R Sybers H D Dood A R & Gerritsen T The Zellweger Syndrome (cerebro hepato renal syndrome) *Birth Defects Original Article Series V* 2 144 1969

17 Rule A H Baran D T & Shwachman H Quantitative determination of watersoluble proteins in meconium *Pediatrics 45* 847 1970

18 Shwachman H & Kulczycki L L Longterm study of one hundred five patients with cystic fibrosis *Am J Dis Child 96* 6 1958

19 Shwachman H Redmond A & Khaw K T Studies in cystic fibrosis Report of 130 patients diagnosed under 3 months of age over a 20-year period *Pediatrics 46* 335 1970

20 Shwachman H Diamond L K Oski F A & Khaw K T The syndrome of pancreatic insufficiency and bone marrow dysfunction *J Paediatr 65* 645 1964

21 Stephan U Busch E W & Dannemann R Ein neuer Screening Test auf Mukoviszidose *Padiat Prax 12* 487 1973

22 Stephan U Busch E W Hellsing K & Kollberg H Cystic fibrosis detection by means of a test strip *Pediatrics* In press

23 Thorner R M & Remein Q R Principles and procedures in the evaluation of screening for disease *Public health monograph* No *67* *Public health service publication* No *846* United States Govern ment Printing Office Washington 25 D C 1961

24 Warwick W J & Pogue R E Computer studies in cystic fibrosis *In* D Lawson (ed) *Proceedings of the 5th International CF Conference* C Nicholls & Co Ltd London 1969 p 320

25 Wilson J M G & Hilleboe H E *Mass health examinations* Public health papers 45 World Health Organization Geneva 1971

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CARBOHYDRATE INTOLERANCE IN INFANTS WITH ACUTE DIARRHOEA AND ITS COMPLICATIONS

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ABSTRACT Chandrasekaran R Kumar V Walia B N S and Moorthy B (Department of Paediatrics Postgraduate Institute of Medical Education and Research Chandigarh India) Carbohydrate intolerance in infants with acute diarrhoea and its complications Acta Paediatr Scand 64 483 1975.—Two hundred and seventy-one infants with acute diarrhoea were studied for the presence of carbohydrate malabsorption and 110 infants (40 6%) were found to have carbohydrate intolerance Malnutrition and severe diarrhoea were found to increase the predisposition to carbohydrate intolerance The incidence of major complications protracted diarrhoea and mortality were significantly higher in the carbohydrate intolerant infants as compared to those with carbohydrate tolerance.

KEY WORDS Acute diarrhoea carbohydrate intolerance malnutrition protracted diarrhoea

Acute diarrhoea an important cause of morbidity in infants and children is often complicated by secondary disaccharide intolerance (23 24 31) Amongst disaccharides lactose intolerance is the most frequent (14) Milk the principle food of infants has a high lactose content (11 22) The milk when given during rehabilitation of diarrhoea may worsen the symptoms if lactose intolerance exists Recognition of this sugar malabsorption promises to be of great practical importance to infants as the exclusion of offending sugar may hasten the rehabilitation from acute diarrhoea (14 18 23 27 33)

This prospective study of 271 infants from Chandigarh emphasises the frequent occurrence of secondary sugar intolerance following acute gastroenteritis both in well nourished as well as in malnourished infants Studies in the past have focused on the frequent occurrence of lactose intolerance following diarrhoea in malnourished infants and those with chronic diarrhoea (23 27 33) However the occurrence of sugar malabsorption in well nourished infants following acute diarrhoe has not been adequately emphasised Th study focuses on the high incidence of con plications and mortality in infants with sug malabsorption

The difficulties in the management carbohydrate intolerance in the developi countries where ready made sugar free fo mulae are either not avilable or are very ex pensive are discussed

MATERIAL AND METHODS

Two hundred and seventy-one infants with acute diarrhoea admitted to the Postgraduate Institute of Medical Education and Research Chandigarh were studied for the presence of carbohydrate intolerance Children above the age of 24 months and those with chronic diarrhoea were excluded All the infants were grouped into various nutritional categories based on weight for age criteria and the presence or absence of oedema (34) The age duration of diarrhoea preceding admission and the nature of antibiotics given were noted Stool microscopic examinations culture for bacteria blood electrolytes and acid–base studies were undertaken

The severity of diarrhoea was graded by a clinical esti

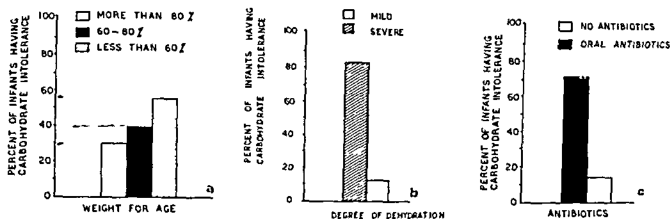


Fig 1 (*a*) Relation of carbohydrate tolerance with nutritional status This shows increasing incidence of carbohydrate intolerance with increasing degree of malnutrition (*b*) Relation of carbohydrate tolerance with the severity of dehydration The incidence of carbohydrate malabsorption is significantly greater in the severely dehydrated infants as compared with those with mild dehydration ($p=<0\ 01$) (*c*) Relation of carbohydrate tolerance with the use of oral antibiotics The incidence of carbohydrate malabsorption is significantly greater in infants where oral antibiotics were used ($p=<0\ 01$)

mate of the degree of dehydration (12) The dehydration was graded as significant if the estimated fluid deficit was more than 5% of the body weight All the infants were given appropriate fluid electrolyte and acid–base corrections Milk feeds were initiated 12–24 hours after the correction of the fluid imbalance and full strength milk was given after 48 hours After the introduction of milk stool pH and sugar were estimated on more than one sample of the fluid part of the stool

Urinary contamination was prevented by the use of urine collectors Stool pH was estimated by the use of nitrazine paper (range pH 4–8) and stool sugar by clinitest tablets (Ames Co Div of Miles Labs Elkhart Ind USA) In all instances of disaccharide intolerance where there was no improvement on lactose exclusion the total reducing substances in the stool were estimated after acid hydrolysis (to detect sucrose intolerance) A diagnosis of carbohydrate intolerance was suspected if the stools were watery frothy and explosive accompanied by irritability abdominal distension and anal soreness with a stool pH of less than 6 and stool sugar of more than 0 5 g/100 ml (13 14) Further confirmation was made by demonstration of improvement in the character pH and sugar content of the stools following the exclusion of the offending sugars

Infants with carbohydrate intolerance were first given curd[1] which has a low lactose content (curd with lactose content of 2 5–3 g/100 ml) [2] Those who improved on curd were classified as having mild lactose intolerance Infants who could not tolerate curd were classified as having severe lactose intolerance and were put on lactose free formulae (soyabean milk or cereal based formula Nestum) [3] Infants who could tolerate oral glucose electrolyte mixtures only were labelled as having multiple disaccharide intolerance (12) Intolerance to oral monosaccharides necessitating intravenous therapy formed the basis of diagnosis of monosaccharide intolerance (23)

Antibiotics were administered to 95 infants The indications for antibiotic therapy were isolation of pathogens from the stool and presence of systemic infection The complications during hospital stay were grouped as (i) major (ii) minor and (iii) protracted diarrhoeas (2 6 26) Complications such as septicemia disseminated intravascular coagulation (22) non specific enterocolitis pneumatosis intestinalis (7) and hypothermia were grouped as major complications Oral moniliasis anal soreness and fissure in ano were labelled as minor If the duration of diarrhoea after initiation of therapy was more than 2 weeks the diarrhoea was called protracted diarrhoea

OBSERVATIONS

The mean age of 271 infants with acute diarrhoea was 8 7±0 7 (S E M) months The mean duration of diarrhoea before admission was 5 9±5 0 (S D) days Ninety four infants (34 7%) were well nourished 107 infants (39 5%) were undernourished and 70 (25 8%) were marasmic (Fig 1*a*) One hundred and fifty two had significant dehydration and 119 were mildly dehydrated Enteric infections (both bacterial and protozoal) were present in 60 infants Systemic infections were present in 60 and no infections were diagnosed in 151 infants

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[1] Curd or *Dahi* is a sour thick and coagulated milk product bearing a close resemblance to Yoghurt Lacticacid formation is the basis underlying the production of curd
[2] Lactose content of the curd used in Pgimer Chandigarh was analysed for lactose content by micromethod (29)
[3] Nestle s Products (India) Ltd New Delhi 110001 Reconstituted Nestum provides approximately 47 calories and 3 2 g of protein per 100 ml

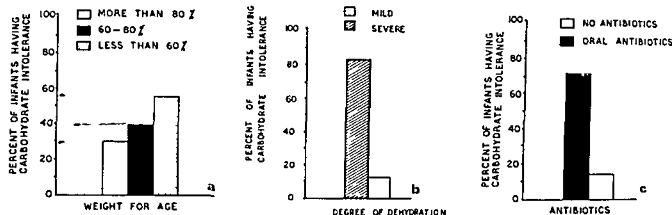


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carbohydrate intolerance may be difficult to make If the clinical response to the exclusion of offending sugar is favourable the diagnosis of sugar malabsorption should be acceptable As there was not a single instance of carbohydrate intolerance with stool sugar less than 0 5 g/100 ml and all instances where stool sugar was more than 0 5 g/100 ml were associated with carbohydrate intolerance stool sugar by clinitest tablets appears to be a better test for diagnosis of carbohydrate intolerance

Infants with diarrhoea and carbohydrate intolerance have a higher mortality (21 8%) than those without intolerance (4 9%) Infants with carbohydrate intolerance have a high incidence of protracted diarrhoea and major complications such as septicemia disseminated intravascular coagulation non specific enterocolitis and pneumatosis intestinalis The mortality in infants with carbohydrate intolerance was unrelated to the above factors in this study

As lactose free formulae and carbohydrate free formulae are either not available at all or are prohibitively expensive in developing countries management of these infants is not easy Infants who have multiple carbohydrate intolerance including monosaccharide malabsorption should ideally be treated with parenteral hyperalimentation (6 32) The risk of complications following parenteral hyperalimentation (4 9 16 19) and lack of adequate facilities for its institution and monitoring preclude the widespread use of such a mode of therapy Non availability of a satisfactory carbohydrate free formula was probably responsible for the disappointing results in infants with monosaccharide intolerance in the present study

Seventy six out of 90 infants with lactose intolerance could be successfully rehabilitated with curd which has a lower lactose content than milk Curd can be prepared at home in tropical climates it can be easily homogenised and fed by bottle Infants who do not tolerate curds (severe lactose intolerance) may need to be fed with soya milk or Nestum Infants who have multiple disaccharide intolerance can be given soya bean milk with added glucose in place of sucrose It is hoped that mortality and morbidity from acute diarrhoea will decrease as a consequence of vigilance for the presence of carbohydrate intolerance and the adoption of an early methodical approach towards the rehabilitation of these infants

REFERENCES

1 Auricchio S & Rubino A Intestinal glycosidase activities in congenital malabsorption of disaccharides *J Pediatr* 66 55 1965
2 Avery G B Villavivencio O Lily J R & Randalph J C Intractable diarrhoea in early infancy *Pediatrics* 41 712 1968
3 Bayless T M & Shi Shuanghuang Inadequate Intestinal digestion of lactose *Am J Clin Nutr* 22 250 1969
4 Behrman R E Total intravenous alimentation Forward *J Pediatr* 81 127 1972
5 Burke V & Dankes D M Monosaccharide malabsorption in young infants I *Lancet* I 1177 1966
6 Charles J Hyman M D Reiter J Rodman J & Drash A L Parenteral and oral alimentation in the treatment of non specific protracted diarrhoeal syndrome of infancy *J Pediatr* 78 17 1971
7 Coello-Ramirez P Gutierrez Topete G & Lifshitz F Pneumatosis intestinalis *Am J Dis Child* 3 120 1970
8 Davidson M Disaccharide intolerance *Pediatr Clin North Am* 14 93 1967
9 Driscoll J M Jr Heird W C Schullinger J N Gangaware R D & Winters R W Total intravenous alimentation in low birth weight infants A preliminary report *J Pediatr* 81 145 1972
10 Elsas L J Hillman R E Patterson H & Rosenberg L E Renal and intestinal hexose transport in familial glucose galactose malabsorption *J Clin Invest* 49 576 1970
11 Emmett Holt L Jr *Pediatrics* (ed H L Barnett) Appleton-Century-Crofts Educational Division/Meredith Corporation 15th edition New York 1972 p 158
12 Ghai O P & Kumar V Assessment of weight loss due to fluid in dehydration due to diarrhoea *Pediatr Clin India* 4 273 1969
13 Ghai O P Kumar L & Kumar V Disaccharide intolerance in malnutrition *Indian Pediatr* 6 364 1969
14 Gracey M & Burke V Sugar induced diarrhoea in children *Arch Dis Child* 48 331 1973
15 Harries J T & Francis D E M Temporary monosaccharide intolerance *Acta Paediatr Scand* 57 505 1968
16 Helmuth W V Adam P A J & Sweet A Y The effect of protein hydrolysate monosaccharide infusion on low birth weight infants *J Pediatr* 81 129 1972

Table 1 *Incidence of complications and mortality in the different nutritional groups in carbohydrate intolerant and tolerant infants*

	Complications			
	Minor	Major	Protracted diarrhoea	Mortality
Carbohydrate intolerants				
Well nourished	3	1	1	0
Under nourished	6	7	10	7
Marasmus	10	17	14	17
Total	19	25	25	24
Carbohydrate tolerants				
Well nourished	0	0	0	2
Under nourished	1	2	0	3
Marasmus	1	3	1	3
Total	2	5	1	8

The incidence of complications protracted diarrhoeas and mortality was significantly higher among the carbohydrate intolerant group as compared with the tolerant group ($p=<0\ 01$) Twenty six infants had protracted diarrhoea, 25 of these were of the intolerant group (22 7%) Out of these 25 cases 6 had multiple disaccharide intolerance and 5 had severe lactose intolerance The incidence of protracted diarrhoea was greater in the malnourished infants with carbohydrate intolerance Among the 30 cases who had major complications 25 occurred in the intolerant group and 5 in the tolerant group Seventeen infants had systemic sepsis 6 had disseminated intra vascular coagulation 3 had non specific enterocolitis 2 had hypothermia and 2 had pneumatosis intestinalis Both major and minor complications were significantly greater among the carbohydrate intolerants ($p=<0\ 01$)

Twenty four of the total 32 deaths occurred in the carbohydrate intolerant group The incidence of mortality among the intolerants was found to increase with increasing degree of malnutrition Of the 24 deaths in the intolerant group, 17 occurred in infants with marasmus and 7 in the undernourished group No deaths occurred in the well nourished group (Table 1)

The 8 instances of death among the carbohydrate tolerants were due to the underlying systemic infections of which the diarrhoea was probably a symptom The mean duration of diarrhoea in the carbohydrate intolerants was 10 4±6 9 (S D) days while it was only 3 0±2 5 (S D) days among the tolerants ($p=<0\ 01$)

DISCUSSION

Carbohydrate intolerance is a frequent complication of acute gastroenteritis in infants all over the world (14 24) The total incidence of carbohydrate intolerance was 40 6% in the present study Lifshitz et al (23) have reported an incidence of 77% The higher incidence of carbohydrate intolerance in their study from Mexico may be related to the inclusion of larger number of infants with malnutrition and chronic diarrhoea As in other studies this report demonstrates a high incidence of lactose intolerance as compared to other sugars When the carbohydrate intolerant infant does not improve on lactose exclusion the underlying problem is likely to be multiple disaccharide intolerance or rarely monosaccharide malabsorption (14 24) Malnourished infants infants with prolonged diarrhoea and those who have severe diarrhoea are more likely to develop sugar intolerance

Elaborate diagnostic procedures such as the estimation of disaccharidase activities from jejunal mucosa (1 33) studies of uptake of sugar by jejunal mucosa (10) and oral sugar tolerance tests (17 18) are complicated and unnecessary (14) Tests such as stool sugar (14 20 23) stool pH (8 23 32) and the evaluation of clinical response to the challenge and exclusion of the suspected sugars are simple and practical (12 25) If the patient develops frothy watery and explosive diarrhoea following milk administration or oral sugar feeding and if the stool pH falls below 6 and stool sugar is more than 0 5 g/100 ml the presence of carbohydrate intolerance is very likely When the stool pH is 6 and stool sugar is 0 5 g/100 ml the diagnosis of

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POLYUNSATURATED FATTY ACID LIPIDOSIS

II *Lipid biochemical studies*

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ABSTRACT Svennerholm L, Hagberg B Haltia M Sourander, P and Vanier M T (*Departments of Neurochemistry Paediatrics and Pathology I University of Gothenburg Sweden and Pathology II University of Helsinki Finland*) Polyunsaturated fatty acid lipidosis (PFAL) Acta Paediatr Scand 64 489 1975 —Lipid analyses were performed on cerebral tissue from three children who had died of a progressive encephalopathy and from one living child in an early stage of the disease In the terminal stage of the disease the cortex and white matter content of all lipid classes particularly the sphingolipids, were very low The concentration of gangliosides of the cerebral cortex was 10% and of cerebrosides in white matter 2–3% of the normal values for the age The porportion of the minor gangliosides with short carbohydrate chains was increased because the reduction affected mainly the four major brain gangliosides G_{M1} G_{D} G_{D1b} and G_{T1} In the child from whom the biopsy specimen was obtained in an early phase of the disease the cerebral lipid pattern appeared to be normal A patient who had died of neuronal ceroid lipofuscinosis (Jansky Bielschowsky) did not show any major lipid changes The fatty acid patterns of the phosphoglycerides showed such changes as have never been observed in any other disease In the three advanced cases the fatty acid compositions in cerebral cortex and white matter were identical In ethanolamine phosphoglycerides the proportions of 18 1 and 20 4 (*n*-6) were increased while those of 22 4 (*n*-6) and 22 6 (*n* 3) were markedly diminished Similar changes in the fatty acid patterns were found in the other phosphoglycerides In the early phase of the disease 22 4 (*n*-6) was decreased and 18 1 increased We propose that this new disease be termed polyunsaturated fatty acid lipidosis (PFAL)

KEY WORDS Polyunsaturated fatty acid lipidosis (PFAL) infantile progressive encephalopathy phosphoglycerides, gangliosides cerebrosides fatty acid composition dokosahexaenoic acid

At examination of a case of infantile progressive encephalopathy with disturbed polyunsaturated fat metabolism (5) we demonstrated profound biochemical alterations in the cerebral cortex and white matter The concentrations of the lipids were very low particularly those of the glycosphingolipids gangliosides cerebrosides and sulfatides Such a severe diminution of cerebral lipids has only been found once before and that was in a case described as congenital amaurotic idiocy (4) In that case there was also as in cases of the present disease very severe neuronal destruction In the first case of the new disease the fatty acid compositions of the phosphoglycerides revealed a remarkably low concentration of dokosahexaenoic acid 22 6 (*n* 3) which is the major fatty acid derived from linolenic acid The pattern of the other major polyunsaturated fatty acids were also changed with an increase in arachidonic acid 20 4 (*n*-6) and a decrease in 22 4 (*n* 6)

Since the first observations were made in a single case it was considered desirable to find

17 Holzel A Mereu T & Thompson M L Severe lactose intolerance in infancy *Lancet II* 1346 1962
18 Holzel A Schwarez V & Sutcliff K W Defective lactose absorption causing malnutrition in infancy *Lancet I* 1126 1959
19 Johnson J D Albritton W L & Sunshine P Hyperammonaemia accompanying parenteral nutrition in new born infants *J Pediatr 81* 129 1972
20 Kerry K R & Anderson C M A ward test for sugar in faeces *Lancet I* 984 1964
21 Kumar V Sarup B M & Narang A Disseminated intravascular coagulation in children *Indian Pediatr 10* 493 1973
22 Laupus W E In *Textbook of Pediatrics* W E Nelson (ed) W B Saunders Co Philadelphia 1968 9th edition p 151
23 Lifshitz F Coello-Ramirez P Gutierrez Topete G & Maria Cinta Cornado-Cornet Carbohydrate intolerance in infants with diarrhoea *J Pediatr 79* 760 1971
24 Lifshitz F Coello-Ramirez P & Contreras Gutierroz M The response of infants to carbohydrate oral loads after recovery from diarrhoea. *J Pediatr 79* 612 1971
25 Lifshitz F Coello-Ramirez P & Gutierrez Topete G Monosaccharide intolerance and hypoglycaemia in infants with diarrhoea I Clinical course of 23 infants *J Pediatr 77* 595 1970
26 Lloyd-Still J D Schwachman H & Filler R M Protracted diarrhoea of infancy treated by intravenous alimentation I Clinical studies in 16 infants *Am J Dis Child 125* 358 1973
27 McMichael H B Webb J & Dawson A M Lactase deficiency in adults A cause of functional diarrhoea *Lancet I* 717 1965
28 Nicholls L *Tropical Nutrition and Dietetics* Bailliere Tindall & Cox London 1961 4th edition p 23
29 Oser B L *Hawke s Physiological Chemistry* Tata-McGraw Hill Publishing Co Ltd New Delhi 1965 14th edition p 392
30 Schwachman H Lloyd J D Con Taik Khaw & Antonwiz I Protracted diarrhoea of infancy treated by intravenous alimentation II Studies of intestinal biopsy results *Am J Dis Child 125* 365 1973
31 Soeparto P Stobo E A & Walker Smith J A Role of chemical examination of the stool in diagnosis of sugar malabsorption in children *Arch Dis Child 47* 56 1972
32 Sunshine P & Kretchmer N Studies of small intestine during development III Infantile diarrhoea associated with intolerance to disaccharides *Pediatrics 34* 38 1964
33 Weijers H A van de Kamer J H Dicke W K & Ijsseling J Diarrhoea caused by deficiency of sugar splitting enzymes II *Acta Paediatr Scand 50* 55 1961
34 Wellcome Trust sponsored international working party Classification of infantile malnutrition *Lancet II* 302 1970

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Table 2 *Composition of cerebral white matter*

Values are expressed as μmol/g fresh tissue except for water content (%) and individual phospholipids (mole %). The numbers in parentheses denote the number of analyses

	n	Controls (3–16 years) Mean	Controls (3–16 years) S.D.	Polyunsaturated fatty acid lipidoses Å.N. 2.6 y	M.L. 6.0 y	T.N. 8.5 y	T.A. 10.3 y	Jansky Bielschowsky T.H. (5.2 y) Total brain
Water	(18)	70.1	1.9	–	91	88	–	–
Cholesterol	(13)	121.4	11.8	–	14	12	15	60
Phospholipids	(18)	112.3	9.7	65	19	24	24	99
EPG	(7)	35.4	2.1	31	29	29	31	–
SPG	(7)	19.1	2.3	0	12	11	12	–
IPG	(7)	2.2	0.9	1	5	3	2	–
CPG	(7)	27.0	1.3	33	33	38	38	–
Sph	(7)	16.3	1.3	16	21	19	18	–
Lipid NANA	(8)	0.94	0.16	–	0.47	0.27	0.29	2.27
Cerebrosides	(7)	47.2	3.6	22	1.0			–
Sulfatides	(7)	11.2	2.3	7	0.8			–

In the cerebral white matter (Table 2) the three terminal cases showed extremely low values for all the lipids and only insignificant differences from the lipid pattern of cerebral cortex. The phospholipid concentration was 25% of controls, cholesterol 12% and the cerebrosides and sulfatides only 2%. The phospholipid pattern was the same in white matter as in the cerebral cortex. The ganglioside concentration and pattern of individual gangliosides were also the same in white matter as in the cortex. The biopsy specimen from Case Å.N. had a slightly smaller total phospholipid content but the phospholipid pattern did not differ significantly from that of the controls. The cerebroside concentration was about 50% of normal. The concentration of ganglioside could not be determined because of lack of material. Thin layer chromatograms of lipids in the biopsy specimen of unseparated grey and white matter from case T.H. with late infantile amaurotic idiocy were normal. Quantitative analysis of this specimen was unreliable because of dehydration of the material.

Concentrations of individual gangliosides

Thin layer chromatograms of the total gangliosides developed in propanol–water 3:1 v/v and chloroform–methanol–water 60:32:7 v/v showed very similar ganglioside patterns in all the three chronic cases. The patterns were the same in cerebral cortex and white matter. The chromatograms showed a pronounced diminution of particularly gangliosides G_{D1a} and G_{M1} and, though to a lesser extent, of G_{D1b} and G_{T1}. Because of the pronounced diminution of the major brain gangliosides the relative concentrations of the minor gangliosides were much higher than usual. The thin layer plates left the impression that particularly mono- and disialosyllactosylceramides G_{M3} and G_{D3} were markedly increased but quantitative determinations in Case M.L. showed that their concentrations, expressed in nmol/g wet weight, were within the normal range (Table 3). The thin layer chromatogram of the brain specimen from Case T.H. with neuronal ceroid lipofuscino-

Table 3 *The concentration of gangliosides in cerebral cortex and white matter*

Ganglioside	Controls (n = 7) 2 months–2 years C.C.	Wh.m.	Case M.L. 6.0 years C.C.	Wh.m.
G_{M3}	30*	22	34	51
G_{D3}	41	20	36	41
G_{M2}	84	60	33	34
G_{D2}	72	19	11	23
G_{M1}	438	373	50	73
G_{D1a}	502	172	20	34
G_{D1b}	106	35	27	34
G_{T1}	103	32	16	16

Values are expressed in nmoles/g wet weight

Table 1 *Lipid composition of cerebral cortex*

Values are expressed in μmol/g wet weight except for water (%) and individual phospholipids (mole %) The numb in parentheses denote the number of analyses

	n	Controls (3–16 years)		Polyunsaturated fatty acid lipidoses			
		Mean	S D	Å N 2 6 y	M L 6 0 y	T N 8 5 y	T A. 10 3 y
Water	(17)	84 0	1 8	–	92	89	90
Cholesterol	(10)	28 2	3 3	–	9	9	12
Phospholipids	(17)	47 1	6 6	43	14	18	18
EPG	(6)	35 3	0 8	–	30	29	29
SPG	(6)	14 6	1 1	–	13	13	15
IPG	(6)	3 0	1 0	–	3	4	1
CPG	(6)	37 0	1 5	–	35	37	37
Sph	(6)	10 2	0 8	–	19	18	19
Lipid NANA	(12)	2 68	0 35	–	0 30	0 25	0 23

out whether all the biochemical findings could be confirmed in new cases with the same clinical and morphological features In the present study we have made analysis of the lipids in two further Swedish cases (3) and in autopsy material of one case with long standing course representative for the Finnish series of patients published under the heading of the infantile type of so called neuronal ceroid lipofuscinosis (6, 7 15)

MATERIAL AND METHODS

Clinical material

Swedish cases Autopsy specimens of brain and liver were available from Case M L aged 6 0 yrs Case T N aged 8 5 yrs and brain biopsy material taken at the age of 2 6 yrs from Case Å N (3)

Finnish case The autopsy material from Case T A aged 10 3 yrs consisted of the right frontal lobe A clinical and morphological report of this case has been published previously (6) For comparison a small biopsy specimen from a 5 2 year old child T H with neuronal ceroid lipofuscinosis (Jansky Bielschowsky) was also analysed at the same time The specimens had been stored at −20° wrapped in polyethene films prior to analysis and except for the biopsy sample of Case T H which only weighed 23 mg they had not lost any measurable quantities of water during storage The specimens were separated into grey and white matter except for the one from T H which was analysed unseparated

The control material consisted of brain specimens from 18 children aged 3–16 years Fourteen of the children were killed in traffic accidents or had drowned The remaining 4 children had died of diseases with no primary involvement of CNS None of the children had any previous record of mental or neurological impairment Routine histological examination of brain was normal

Methods

The quantitative methods used for the lipid analyses were the same as those used by Svennerholm & Vanier (20) for analysis of the lipid composition of normal human brain The fatty acid composition of the phosphoglycerides was determined by gas–liquid chromatography (17) The quantitative determination of gangliosides were performed in the same way as in an earlier investigation of gangliosides of infant brain (22) The ganglioside nomenclature by Svennerholm (18) has been used

RESULTS

Lipid composition

In the terminal stage of the new disease the cerebral cortex was very lipid poor and the concentrations of phosphoglycerides and cholesterol were only 40% of those in age matched controls (Table 1) The phospholipid pattern showed a significant reduction in ethanolamine phosphoglycerides and a significant increase in sphingomyelins The ganglioside concentration expressed as lipid NANA was extremely low (10% of normal) The two Swedish cases and the Finnish case did not show any significant difference the slightly higher figure for lipid NANA in Case M L might be explained by the shorter duration of the disease The biopsy specimen from Case Å N showed normal values for total phospholipids the lipid pattern also appeared normal at thin layer chromatography No further analyses were performed because of shortage of material

Table 2 *Composition of cerebral white matter*

Values are expressed as μmol/g fresh tissue except for water content (%) and individual phospholipids (mole %) The numbers in parentheses denote the number of analyses

	n	Controls (3–16 years) Mean	S D	Polyunsaturated fatty acid lipidoses Å N 2 6 y	M L 6 0 y	T N 8 5 y	T A 10 3 y	Jansky Bielschowsky T H (5 2 y) Total brain
Water	(18)	70 1	1 9	–	91	88	–	–
Cholesterol	(13)	121 4	11 8	–	14	17	15	60
Phospholipids	(18)	112 3	9 7	65	19	24	24	99
EPG	(7)	35 4	3 1	35	29	29	31	–
SPG	(7)	19 1	2 3	20	12	11	12	–
IPG	(7)	2 2	0 9	1	5	3	2	–
CPG	(7)	27 0	1 3	33	33	38	38	–
Sph	(7)	16 3	1 3	16	21	19	18	–
Lipid NANA	(8)	0 94	0 16	–	0 47	0 27	0 29	2 27
Cerebrosides	(7)	47 2	3 6	22	1 0			–
Sulfatides	(7)	11 2	2 3	7	0 8			–

In the cerebral white matter (Table 2) the three terminal cases showed extremely low values for all the lipids and only insignificant differences from the lipid pattern of cerebral cortex The phospholipid concentration was 25% of controls cholesterol 12% and the cerebrosides and sulfatides only 2% The phospholipid pattern was the same in white matter as in the cerebral cortex The ganglioside concentration and pattern of individual gangliosides were also the same in white matter as in the cortex The biopsy specimen from Case Å N had a slightly smaller total phospholipid content but the phospholipid pattern did not differ significantly from that of the controls The cerebroside concentration was about 50% of normal The concentration of ganglioside could not be determined because of lack of material Thin layer chromatograms of lipids in the biopsy specimen of unseparated grey and white matter from case T H with late infantile amaurotic idiocy were normal Quantitative analysis of this specimen was unreliable because of dehydration of the material

Concentrations of individual gangliosides

Thin layer chromatograms of the total gangliosides developed in propanol–water 3 1 v/v and chloroform–methanol–water 60 32 7 v/v showed very similar ganglioside patterns in all the three chronic cases The patterns were the same in cerebral cortex and white matter The chromatograms showed a pronounced diminution of particularly gangliosides G_{D1a} and G_{M1} and though to a lesser extent of G_{D1b} and G_{T1} Because of the pronounced diminution of the major brain gangliosides the relative concentrations of the minor gangliosides were much higher than usual The thin layer plates left the impression that particularly mono- and disialosyllactosylceramides G_{M3} and G_{D3} were markedly increased but quantitative determinations in Case M L showed that their concentrations expressed in nmol/g wet weight were within the normal range (Table 3) The thin layer chromatogram of the brain specimen from Case T H with neuronal ceroid lipofuscino

Table 3 *The concentration of gangliosides in cerebral cortex and white matter*

Ganglioside	Controls (n=7) 2 months–2 years C C	Wh m	Case M L 6 0 years C C	Wh m
G_{M3}	30*	72	34	51
G_{D3}	41	70	36	41
G_{M2}	84	60	33	34
G_{D2}	22	19	11	23
G_{M1}	438	373	50	73
G_{D1a}	502	172	70	34
G_{D1b}	106	35	27	34
G_{T}	103	32	16	16

Values are expressed in nmoles/g wet weight

Table 4 *Fatty acid composition of ethanolamine phosphoglycerides*

Values are weight percentage of methyl esters The following fatty acids were detected in small amounts (in gener less than 1%) and were added to the other values to make a total of 100% 18 2 (*n*-6) 18 3 (*n*-6) 20 3 (*n* 9) 2? (*n* 3) 24 4 (*n* 6)

	Cerebral cortex						Cerebral white matter					
	Controls (*n*=11) 2–10 y		PFAL				Controls (*n*=9) 2–10 y		PFAL			
	M	S D	Å N 2 6 y	M L 6 0 y	T N 8 5 y	T H 10 3 y	M	S D	Å N 2 6 y	M L 6 0 y	T N 8 5 y	T H 10 3 y
Fatty acid												
16 0	5 7	0 8	8	12	9	11	4 3	0 4	6	10	12	10
16 1	0 6	0 1	2	4	3	2	0 8	0 3	1	3	2	2
18 0	29 0	2 1	23	9	7	7	8 7	1 2	12	8	9	6
18 1	9 3	0 9	16	38	38	42	34 1	2 8	33	38	38	44
20 1	0 5	0 4	1	1	1	1	6 0	0 9	3	1	1	[illegible]
20 3 (*n* 6)	1 5	0 6	2	1	1	1	2 5	0 5	3	1	1	1
20 4 (*n*-6)	16 1	1 3	15	24	27	23	10 8	0 7	17	23	24	22
22 4 (*n* 6)	10 0	1 5	7	3	5	4	21 4	2 7	13	4	5	4
22 5 (*n*-6)	2 2	0 8	2	3	2	2	1 2	0 4	1	5	2	[illegible]
22 6 (*n* 3)	23 6	2 4	24	2	3	5	6 5	0 8	9	2	4	7
Saturated acids	34 7	2 7	31	21	16	17	13 0	1 6	17	18	20	15
Monoenoic acids	10 4	0 8	19	42	41	46	40 8	3 7	38	42	41	48
Linoleic acid series	30 5	3 3	25	31	38	31	37 6	3 3	35	34	35	[illegible]9
Linolenic acid series	24 2	2 4	24	3	3	6	7 4	0 7	9	3	4	7

sis Jansky Bielschowsky type showed a normal ganglioside pattern

Fatty acid composition The most striking findings in the brains from the 3 patients who had died from the disease were the great similarities between the fatty acid patterns of cerebral cortex and white matter and the pronounced diminution of fatty acids of the linolenic acid series (Tables 4–6) In cerebral cortex the proportions of the monoenoic fatty

Table 5 *Fatty acid composition of serine phosphoglycerides*

Values are weight percentage of methyl esters The following fatty acids were detected in small amounts (in general less than 1%) and were added to the other values to make a total of 100% 18 2 (*n*-6) 18 3 (*n* 6) 20 3 (*n* 9) ?? 5 (*n* 3) 24 4 (*n* 6)

	Cerebral cortex						Cerebral white matter					
	Controls (*n*=11) 2–10 y		PFAL				Controls (*n*=9) 2–10 y		PFAL			
	M	S D	Å N 2 6 y	M L 6 0 y	T N 8 5 y	T H 10 3 y	M	S D	Å N 2 6 y	M L 6 0 y	T N 8 5 y	T H 10 3 y
Fatty acid												
16 0	4 6	1 9	4	7	7	8	2 7	1 0	3	4	9	6
16 1	0 5	0 2	1	2	1	1	0 4	0 1	<1	1	1	1
18 0	43 3	3 4	41	32	29	29	44 3	2 2	44	32	28	24
18 1	10 1	1 5	17	46	47	49	37 6	1 5	38	45	47	43
20 1	0 4	0 3	<1	3	3	4	3 3	0 6	2	3	3	5
20 3 (*n* 6)	1 4	0 3	1	1	1	<1	1 0	0 2	2	1	1	1
20 4 (*n*-6)	2 5	0 4	4	5	5	5	2 1	0 3	3	5	6	7
22 4 (*n* 6)	5 8	1 1	3	1	2	1	3 4	0 9	2	2	2	2
22 5 (*n* 6)	3 4	1 3	3	1	1	1	0 5	0 3	1	2	1	1
22 6 (*n* 3)	25 6	3 1	25	1	1	1	2 3	0 5	4	1	1	2
Saturated acids	48 1	3 3	45	38	36	37	47 0	1 7	47	37	36	30
Monoenoic acids	10 9	1 4	18	50	51	55	41 3	1 8	41	48	52	49
Linoleic acid series	13 7	2 6	11	10	11	8	8 3	2 1	8	12	11	11
Linolenic acid series	26 9	3 1	26	1	1	1	2 6	0 7	4	3	1	2

Table 6 *Fatty acid composition of choline phosphoglycerides*

Values are weight percentage of methyl esters The following fatty acids were detected in small amounts (in general less than 1%) and were added to the other values to make a total of 100% 18 2 (*n*-6) 18 3 (*n*-6) 20 3 (*n* 9) 22 4 (*n*-6) 22 5 (*n*-6) 22 5 (*n* 3) 24 4 (*n*-6)

	Cerebral cortex						Cerebral white matter					
	Controls (*n*=11) 2–10 y		PFAL				Controls (*n*=9) 2–10 y		PFAL			
	M	S D	Å N 2 6 y	M L 6 0 y	T N 8 5 y	T H 10 3 y	*M*	S D	Å N 2 6 y	M L 6 0 y	T N 8 5 y	T H 10 3 y
Fatty acid												
16 0	45 8	1 9	44	48	49	53	33 9	1 9	38	46	50	51
16 1	3 5	0 9	3	7	4	3	2 8	0 6	4	7	5	5
18 0	17 6	0 6	10	4	2	2	14 6	1 1	11	3	2	1
18 1	26 4	1 3	30	36	37	38	42 6	2 6	41	39	36	37
20 1	0 5	0 1	1	<1	<1	<1	1 2	0 2	1	<1	<1	1
20 3 (*n*-6)	1 2	0 3	1	<1	1	<1	0 6	0 1	1	<1	<1	<1
20 4 (*n*-6)	5 8	0 5	6	3	4	3	3 8	0 4	3	3	4	3
22 6 (*n*-6)	2 1	0 5	3	<1	<1	<1	0 6	0 2	1	<1	<1	<1
Saturated acids	58 4	1 8	54	53	51	55	47 6	2 0	48	49	52	52
Monoenoic acids	30 4	1 2	34	43	42	41	46 7	2 3	46	46	41	43
Linoleic acid series	8 8	1 1	8	4	7	4	4 7	0 8	4	4	7	4
Linolenic acid series	2 2	0 4	3	<1	<1	<1	0 7	0 1	1	<1	<1	1

acids were strongly increased while stearic acid was the only saturated fatty acid that was diminished The proportion of dokosahexaenoic acid 22 6 (*n* 3)—the major metabolite of linolenic acid—was reduced to less than 1% in serine phosphoglycerides and to 2–5% in ethanolamine phosphoglycerides compared with about 20% or more in the normal brain The proportion of fatty acids of the linoleic acid series was the same in the patients as in the controls but the range of variation of the levels of the individual fatty acids was wide Arachidonic acid 20 4 (*n*-6) was significantly increased in ethanolamine and serine phosphoglycerides while 22 4 (*n*-6) was markedly decreased In choline phosphoglycerides 22 6 (*n* 3) and 20 4 (*n* 6) were diminished The fatty acid data in Case Å N differed from those in the 3 chronic cases The concentration of 22 6 (*n* 3) acid was normal in all the three phosphoglycerides but all the other fatty acids showed the same trend as in the chronic cases —the proportion of monoenoic acids was increased and those of 22 4 (*n*-6) and 22 5 (*n*-6) diminished

In the white matter the fatty acid changes were less striking since the white matter normally has a much higher concentration of monoenoic acids than the cerebral cortex and a lower concentration of polyenoic acids In the 3 long standing cases the proportions of 22 6 (*n* 3) and 22 4 (*n* 6) were diminished while that of 20 4 (*n*-6) was increased in all three phosphoglycerides The increase was particularly evident in ethanolamine phosphoglyceride which normally has a much larger proportion of 20 4 (*n*-6) than the other two phosphoglycerides The diminution of stearic acid was particularly prominent in choline phosphoglycerides In Case Å N the concentration of 22 6 (*n* 3) was higher than in the control brains but that of 20 4 (*n*-6) was much higher and 22 4 (*n*-6) much lower than in the control brains Case Å N thus showed similar but less pronounced changes in the fatty acids of the linoleic acid series than did the long standing cases

DISCUSSION

Clinical and morphological studies of three Swedish cases with a severe progressive encephalopathy (3) and a large number of cases of

a disease seen in Finland and described as an infantile form of neuronal ceroid lipofuscinosis (INCL) (6 7 15) suggested that the Swedish and Finnish cases were one and the same nosological entity The present biochemical study produced further evidence for this concept

The name neuronal ceroid lipofuscinoses was suggested by Zeman et al (24) for three forms of amaurotic familial idiocy late infantile (Jansky Bielschowsky) juvenile (Spielmeyer Sjogren) and adult (Kufs Hallervorden) Zeman et al (24) considered the demonstration of a large accumulation of autofluorescent lipopigments in neuronal ceroid lipofuscinoses to provide a compelling argument for a clearcut nosological separation of the neuronal ceroid lipofuscinoses from other progressive encephalopathies They also emphasized that in the various forms of neuronal ceroid lipofuscinoses no definite abnormalities have been found in the concentrations of the sphingolipids which is the only lipid class that has been thoroughly investigated so far Zeman and associates (24) made a contradictory diagnosis when including our first case (5) among the ceroid lipofuscinoses since we demonstrated several lipid biochemical changes which have never been observed in neuronal ceroid lipofuscinoses Haltia and Santavuori and their associates (6 7 15 16) distinguished the present condition from the previously known progressive encephalopathies but adopted the designation infantile type of so called neuronal ceroid lipofuscinoses Since we have now shown that this form of progressive encephalopathy differs not only clinically and histopathologically but also biochemically from the previous three forms of neuronal ceroid lipofuscinoses it would be incorrect to include it among ceroid lipofuscinoses It is however difficult to suggest a name for the new disease in which the etiology is unknown and only a few aspects of the pathogenesis are known Since severe changes have been demonstrated in the polyunsaturated fatty acid compositions of the phosphoglycerides in brain and serum (3) we have proposed that this new disease be termed polyunsaturated fatty acid lipidosis (PFAL) although we have not been able to show any accretion of polyunsaturated fatty acids or diminished activity of an enzyme capable of degrading these fatty acids

Since our first biochemical study (5) of this disease biochemical determinations have been performed in two studies from Finland Haltia et al (6) found low concentrations of total gangliosides in biopsy specimens from the cerebral cortex in 9 cases The ganglioside pattern showed slight changes with an increase in ganglioside G_{M3} Nevalainen et al (11) studied autopsy material from two cases The study was extensive but the values are difficult to interpret because some of the values in the one control case fell outside the accepted normal range They found a decrease in cholesterol and phospholipids but a relative increase in cerebrosides Many lysosomal enzymes were increased two to five fold in the white matter It is evident that these authors were unable to detect the very severe lipid reduction found in our previous (5) and our present investigations

In our material of long standing cases the diminution of gangliosides was very pronounced particularly that of the four major brain gangliosides localised mainly in the synaptosomes (18) We have not found such a low concentration of these gangliosides in any other condition except for a case of congenital amaurotic idiocy (4) We are inclined to ascribe the serious diminution of gangliosides to the extensive loss of cortical nerve cells and their synaptic connections This assumption is supported by another finding namely Ohman s (13) recent observation in our laboratory that the major activity of brain sialidase is confined to the synaptosomes In our Case T N the sialidase activity of cerebral cortex and white matter was approximately only one tenth of normal Since the brain sialidase degrades transferrin in brain and CSF to the τ fraction also the early disappearance of the τ-fraction

from the cerebrospinal fluid (3) indicates that the sialidase activity is reduced early

The very low concentrations of cholesterol and phospholipid in the white matter suggests a severe diminution of the myelin lipids The concentration of the characteristic myelin lipid cerebroside was extremely low and we were able to make only a quantitative determination of it in Case M L from which we had a large amount of tissue In the other two long standing cases thin layer chromatography showed only traces of cerebrosides Cerebrosides occur not only in the myelin but also in oligodendroglial cells (14) and the plasma membranes of neurons [1] The severe reduction of cerebrosides found in our 3 autopsy cases can hardly be accounted for solely by a complete loss of myelin and oligodentroglial cells but probably also by a diminished contribution from neurons

In a previous report (5) we underlined the close similarities between the fatty acid patterns of the phosphoglycerides in the cerebral cortex and white matter and the pronounced diminution of 22 6 (*n* 3) The fatty acid composition of the phosphoglycerides of myelin differs from those of other subcellular fractions (8) In myelin the proportions of monoenoic acids are much larger and that of 22 6 (*n* 3) much smaller In inherited or acquired diseases with severe diminution of myelin (19 23) the fatty acid patterns of the cerebral cortex and white matter are similar since the concentration of monoenoic acids are diminished and that of 22 6 (*n* 3) is increased in the white matter PFAL is an exception in this respect In PFAL the fatty acid patterns of cerebral cortex and white matter are similar because the proportions of monoenoic acids are increased and that of 22 6 (*n* 3) is diminished in the cerebral cortex The increase in monoenoic acid concerns mainly 18 1 The fatty acid 20 1 of ethanolamine phosphoglyceride which seems to be the most characteristic fatty acid of myelin[2] is diminished The porportion of 16 1 is increased but this increase might depend upon the increase in 16 0

That the marked diminution of 22 6 (*n* 3) found in the first case (5) appears to be a characteristic of the disease was corroborated by the same finding in a further two advanced cases A diminution of the same fatty acid was reported in Menke s disease by O Brien & Sampson (12) and by French et al (2) but Lou et al (10) reported that the concentration was normal The validity of the last mentioned report is questionable since the values noted in the controls and the case of Menke s disease were only 25% of what is considered normal (21) In a recent case of Menke s disease analysed with the same method as that used in this study the proportion of 22 6 (*n* 3) was found to be normal [3] Our previous conclusion that an extensive diminution of 22 6 (*n* 3) has been found only in the new disease is still valid It is difficult to interpret this result with our present knowledge about the metabolism of fatty acids of the linolenic acid series The serious reduction of 22 6 (*n* 3) might depend on a disturbed biosynthesis of this fatty acid or a loss of brain structures containing this fatty acid

The latter possibility is strengthened by the finding of Breckenridge et al (1) who showed that 22 6 (*n* 3) was enriched in the synaptosomes Recent studies in our laboratory (9) have not revealed any particular synaptosomal enrichment of 22 6 (*n* 3) and judging from our study of enriched glial and neuronal fractions (8) the proportion of 22 6 (*n* 3) is the same in glial as in neuronal phosphoglycerides

At present it seems more likely that the diminution of 22 6 (*n* 3) is due to a defect of the enzyme systems that desaturate and elongate 18 2 (*n* 3) to 22 6 (*n* 3) These enzyme systems are common to the fatty acids of the linoleic and linolenic acid series and a primary disturbance of the further metabolism of 20 4 (*n*-6) might explain both the formation of

[1] A Hamberger & L Svennerholm unpublished observations
[2] L. Svennerholm & M T Vanier unpublished results
[3] M T Vanier unpublished results

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The name neuronal ceroid lipofuscinoses was suggested by Zeman et al (24) for three forms of amaurotic familial idiocy late infantile (Jansky Bielschowsky) juvenile (Spielmeyer-Sjogren) and adult (Kufs Hallervorden) Zeman et al (24) considered the demonstration of a large accumulation of autofluorescent lipopigments in neuronal ceroid lipofuscinoses to provide a compelling argument for a clearcut nosological separation of the neuronal ceroid lipofuscinoses from other progressive encephalopathies They also emphasized that in the various forms of neuronal ceroid lipofuscinoses no definite abnormalities have been found in the concentrations of the sphingolipids which is the only lipid class that has been thoroughly investigated so far Zeman and associates (24) made a contradictory diagnosis when including our first case (5) among the ceroid lipofuscinoses since we demonstrated several lipid biochemical changes which have never been observed in neuronal ceroid lipofuscinoses Haltia and Santavuori and their associates (6 7 15 16) distinguished the present condition from the previously known progressive encephalopathies but adopted the designation infantile type of so called neuronal ceroid lipofuscinoses Since we have now shown that this form of progressive encephalopathy differs not only clinically and histopathologically but also biochemically from the previous three forms of neuronal ceroid lipofuscinoses it would be incorrect to include it among ceroid lipofuscinoses It is however difficult to suggest a name for the new disease in which the etiology is unknown and only a few aspects of the pathogenesis are known Since severe changes have been demonstrated in the polyunsaturated fatty acid compositions of the phosphoglycerides in brain and serum (3) we have proposed that this new disease be termed polyunsaturated fatty acid lipidosis (PFAL) although we have not been able to show any accretion of polyunsaturated fatty acids or diminished activity of an enzyme capable of degrading these fatty acids

Since our first biochemical study (5) of this disease biochemical determinations have been performed in two studies from Finland Haltia et al (6) found low concentrations of total gangliosides in biopsy specimens from the cerebral cortex in 9 cases The ganglioside pattern showed slight changes with an increase in ganglioside G_{M3} Nevalainen et al (11) studied autopsy material from two cases The study was extensive but the values are difficult to interpret because some of the values in the one control case fell outside the accepted normal range They found a decrease in cholesterol and phospholipids but a relative increase in cerebrosides Many lysosomal enzymes were increased two to five fold in the white matter It is evident that these authors were unable to detect the very severe lipid reduction found in our previous (5) and our present investigations

In our material of long standing cases the diminution of gangliosides was very pronounced particularly that of the four major brain gangliosides localised mainly in the synaptosomes (18) We have not found such a low concentration of these gangliosides in any other condition except for a case of congenital amaurotic idiocy (4) We are inclined to ascribe the serious diminution of gangliosides to the extensive loss of cortical nerve cells and their synaptic connections This assumption is supported by another finding namely Ohman s (13) recent observation in our laboratory that the major activity of brain sialidase is confined to the synaptosomes In our Case T N the sialidase activity of cerebral cortex and white matter was approximately only one tenth of normal Since the brain sialidase degrades transferrin in brain and CSF to the τ fraction also the early disappearance of the τ fraction

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KEY WORDS ^{86}Rb method tissue digoxin concentrations tissue distribution of digoxin myocardial binding of digoxin infants

Clinical experience shows that paediatric patients with congestive heart failure require higher doses of digoxin per unit weight than do adults (22) Studies using tritiated glycoside (11 14) have revealed no significant differences in absorption tissue fixation or excretion between infants and adults which could account for this Based on their findings Dungan et al (11) suggested that the larger doses of digoxin required for digitalization of infants were accompanied by increased blood levels and increased *tissue concentrations of the glycoside* In fact many investigators found that standard maintenance doses of digoxin to infants produce higher plasma concentrations than those obtained in adults (13 25 29 32) The doses *given to infants (>1 month) are 2–5 times larger* on a mg/kg bw basis than those given to adults None the less the differences in plasma concentrations are often small (8 15 17 25 32) However infants seem to tolerate higher plasma concentrations of the glycoside than do adults and show no signs of toxicity at plasma levels that are *definitely toxic in adults* (13 18 30)

Data on the distribution and tissue concentrations of digoxin in paediatric patients are sparse and the question whether there are differences in the tissue binding of digoxin between infants and adults has not been settled In order to further investigate the concentrations of digoxin in different tissues during

ceroid lipofuscin and secondary impairment of the linolenic acid metabolism The study of the brain biopsy specimen obtained from Case Å N in an early phase of the disease showed that the disturbance of 22 6 (*n*-3) is not a primary disorder but probably secondary to a disturbance of the further metabolism of 20 4 (*n* 6) (Table 4)

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REFERENCES

1 Breckenridge W C Gombos G & Morgan I G The lipid composition of adult rat brain synaptosomal plasma membranes *Biochim Biophys Acta* 226 695 1972
2 French J H Sherard E S Lubell H Brotz M & Moore C L Trichopoliodystrophy I Report of a case and biochemical studies *Arch Neurol* 26 229 1972
3 Hagberg B Haltia M Sourander P Svennerholm L & Eeg Olofsson O Polyunsaturated fatty acid lipidosis—infantile form of so-called neuronal ceroid lipofuscinosis I Clinical and morphological aspects *Acta Paediatr Scand 63* 753 1974
4 Hagberg B Hultqvist G Öhman R & Svennerholm L Congenital amaurotic idiocy *Acta Paediatr Scand 54* 116 1965
5 Hagberg B Sourander P & Svennerholm L Late infantile progressive encephalopathy with disturbed polyunsaturated fat metabolism *Acta Paediatr Scand 57* 495 1968
6 Haltia M Rapola J Santavuori P & Keranen A Infantile type of so-called neuronal ceroid lipofuscinosis Part 2 Morphological and biochemical studies *J Neurol Sci 18* 269 1973 *a*
7 Haltia M Rapola J & Santavuori P Infantile type of so called neuronal ceroid lipofuscinosis Histological and electron microscopic studies *Acta Neuropathol (Berl)* 26 157 1973 *b*
8 Hamberger A & Svennerholm L Composition of gangliosides and phospholipids of neuronal and glial cell enriched fractions *J Neurochem 18* 1821 1971
9 Karlsson I Effects of different dietary levels of essential fatty acids on the fatty acid composition of ethanolamine phosphoglycerides in myelin and synaptosomal plasma membranes *J Neurochem* in press
10 Lou H C Holmer G K Reske Nielsen E & Vagn Hansen P Lipid composition in grey and white matter of the brain in Menke s disease *J Neurochem 22* 377 1974
11 Nevalainen T J Panelius M & Riekkinen P J Neuronal ceroid lipofuscinosis Report of two cases with neurochemical and morphological observations *Eur Neurol 9* 298 1973
12 O Brien J S & Sampson E L Kinky hair disease II Biochemical studies *J Neuropathol Exp Neurol 25* 523 1966
13 Öhman R Subcellular fractionation of ganglioside sialidase from human brain *J Neurochem 18* 89 1971
14 Poduslo S E & Norton W T Isolation and some chemical properties of oligodendroglia from calf brain *J Neurochem 19* 727 1972
15 Santavuori P EEG in the infantile type of so-called neuronal ceroid lipofuscinosis *Neuropadiatrie 4* 375 1973
16 Santavuori P Haltia M Rapola J & Raitta C Infantile type of so-called neuronal ceroid lipofuscinosis Part 1 A clinical study of 15 patients *J Neurol Sci 18* 257 1973
17 Svennerholm L Distribution and fatty acid composition of phosphoglycerides in normal human brain *J Lipid Res 9* 570 1968
18 — Ganglioside metabolism *In* M Florkin and E M Stotz (eds) *Comprehensive Biochemistry* vol 18 *Lipid metabolism* Elsevier Amsterdam 1970 pp 201–227
19 Svennerholm L Haltia M & Sourander P Chronic sclerosing panencephalitis II A neurochemical study *Acta Neuropathol (Berl) 14* 284 1970
20 Svennerholm L & Vanier M T The distribution of lipids in the human nervous system II Lipid composition of human foetal and infant brain *Brain Res 47* 457 1972
21 — The distribution of lipids in the human nervous system III Fatty acid composition of phosphoglycerides of human foetal and infant brain *Brain Res 50* 341 1973
22 Vanier M T Holm M Månsson J E & Svennerholm L The distribution of lipids in the human nervous system V Gangliosides and other neutral glycolipids *J Neurochem 21* 1375 1973
23 Vanier M T & Svennerholm L Chemical pathology of Krabbe s disease I Lipid composition and fatty acid patterns of phosphoglycerides in brain *Acta Paediatr Scand 63* 494 1974
24 Zeman W Donahue S Dyken P & Green J The neuronal ceroid lipofuscinosis (Batten Vogt syndrome) *In* P J Vinken and G W Bruyn (eds) *Handbook of clinical neurology* vol *10* North Holland Publishing Company Amsterdam 1970 pp 588–679

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Data on the distribution and tissue concentrations of digoxin in paediatric patients are sparse and the question whether there are differences in the tissue binding of digoxin between infants and adults has not been settled In order to further investigate the concentrations of digoxin in different tissues during

Table 1 *Clinical data on the adult patients*

Pat no	Sex	Age at death	Autopsy diagnosis	Maintenance dosage of digoxin (mg/kg bw 24 h)	Time between digoxin intake and exitus (hours)	Plasma digoxin concentration (ng/ml)	Interval between plasma sampling and death (days)
1	F	66	Myocardial infarction	0 003	–	0 9	5
2	M	75	Myocardial infarction	0 005	15	–	–
3	M	70	Coronary arteriosclerosis	0 002	24	1 1	13
4	M	73	Gastric resection femoral venous thrombosis and pulmonary embolism	0 005	12	1 9	14
5	F	49	Mb Hodgkin Aortic and mitral valvular stenosis aortic valve insufficiency	0 006	10	–	–
6	F	79	Cardiosclerosis	0 006	14	–	–
7	M	85	Gastric ulcer with bleeding	0 003	15	–	–
8	F	67	Carcinoma of the breast	0 004	–	1 9	3
9	M	85	Myocardial infarction pulmonary embolism	0 003	6	–	–
10	M	84	Acute obstruction of the large bowel pneumonia	0 004	22	1 7	2
11	F	91	Generalized atherosclerosis	0 005	36	–	–
12	F	79	Myocardial infarction	0 006	6	1 9	3
13	M	61	Acute appendicitis with peritonitis pulmonary embolism	0 006	2	–	–
14	M	56	Aortic valve stenosis and insufficiency	0 005	18	–	–
15	M	83	Generalized atherosclerosis myocardial infarction	0 004	20	1 8	9
16	M	88	Carcinoma of the lung	0 005	10	–	–
17	F	68	Pulmonary fibrosis	0 005	17	–	–

maintenance therapy tissue analyses have been performed post mortem in 29 patients 12 infants and 17 adults

PATIENTS AND METHODS

Patients

Tissue samples from 29 digoxin treated patients—17 adults and 12 infants—were obtained at autopsy All except 2 neonates (Nos 1 and 2 Table 2) had been on maintenance treatment with digoxin for more than 5 days because of congestive heart failure The two neonates received only the initial oral digitalization dose 0 05 mg digoxin/kg bw/24 h

Table 1 gives the clinical data on the adult patients whose mean age was 74 years (range 49–91) The mean maintenance dose of digoxin (Lanacrist AB Draco Sweden) 0 005 mg/kg bw/24 h was given in one daily dose No patient showed clinical or electrocardiographic signs of digitalis toxicity before or at the time of *exitus* The time between digoxin intake and death averaged 15 h ranging from 2 to 36 h

Table 2 summarizes the clinical data on the paediatric patients whose mean age was 60 days (range 5 days to 8 months) All had serious cardiac malformations Their clinical diagnoses were mostly based on data obtained at cardiac catheterization and angiocardiography and were verified at the post mortem examination The infants were given digoxin (Lanoxin paediatric solution Burroughs Wellcome Ltd UK) according to a prevailing oral dose schedule The daily mean maintenance dose 0 014 mg/kg bw was given at 12 h intervals in 2 equal amounts In no case were signs of digitalis toxicity observed The time between the last dose of digoxin and *exitus* averaged 8 h (range 1 5–12 h)

Tissue digoxin assay

Specimens were taken from the following tissues within 36 h after death heart (atrium and ventricle) kidney (mainly cortex) liver skeletal muscle brain choroid plexus and subcutaneous fat (in adults only) Representative samples of adequate size from areas macroscopically apparently normal could easily be obtained from the different tissues Skeletal muscle specimens were taken from the iliopsoas muscle samples from brain (cerebral hemisphere) contained both grey and white matter The digoxin content in the tissue samples was analysed by means of a modified ^{86}Rb uptake inhibition assay (5) After gentle blotting on filter paper each sample was weighed and after addition of 10 ml 0 9% saline homogenized with an Ultra Turrax homogenizer for 2–4 min in an ice bath Five ml of the homogenate was extracted with 10 ml dichloromethane After centrifugation the dichloromethane phase was transferred to new tubes and the homogenate was similarly extracted for a second time From the first extract samples of various volumes (depending on the concentration of digoxin in the tissue) and from the second 4 ml samples

Table 2 *Clinical data on the infants*

Pat no	Sex	Age at death days (d) months (m)	Autopsy diagnosis	Maintenance dosage of digoxin (mg/kg bw/24 h)	Time between digoxin intake and *exitus* (hours)	Plasma digoxin concentration (ng/ml)	Interval between plasma sampling and death (days)
1	F	5 d	Hypoplastic right ventricle pulmonary valve atresia patent ductus arteriosus		8	–	–
2	M	5 d	Transposition of the great arteries functional single ventricle coarctation of the aorta atrioseptal defect patent ductus arteriosus		9	–	–
3	M	12 d	Interrupted aortic arch	0 011	1 5	1 6	4
4	M	12 d	Transposition of the great arteries coarctation of the aorta atrioventricular defects tricuspid atresia patent ductus arteriosus	0 013	12	–	–
5	M	13 d	Transposition of the great arteries preductal coarctation of the aorta patent ductus arteriosus	0 011	12	–	–
6	F	17 d	Transposition of the great arteries hypoplastic left ventricle interrupted aortic arch	0 012	9	1 6	5
7	F	1 m	Pulmonary valve atresia ventricular and atrioseptal defects patent ductus arteriosus mitral atresia	0 012	7	1 5	1
8	F	2 m	Preductal coarctation of the aorta ventricular septal defect	0 0?0	7	1 9	15
9	M	3 m	Mb Down atrioseptal defect	0 016	12	1 6	8
10	F	3 5 m	Cardiomegaly multiple congenital skeletal and muscular defects	0 015	8	–	–
11	F	4 5 m	Common AV-canal hypoplastic left ventricle	0 017	4	1 2	21
12	F	8 m	Mb Down common AV canal hydronephrosis	0 013	12	2 3	12

not on maintenance therapy see text

were taken and evaporated to dryness in a fume cupboard The samples were then handled as described for plasma digoxin ^{86}Rb assay (5) Each tissue sample was analysed on 2–4 occasions The means of these values are given

To test the method heart and skeletal muscle from nondigitalized patients were analysed No inhibition of the ^{86}Rb uptake in erythrocytes was produced by extracts from these tissues In experiments where known amounts of digoxin were added to homogenized skeletal muscle a recovery of 93 6±3 5% was obtained

In some of the adult and paediatric patients plasma digoxin concentrations during maintenance therapy had been analysed by means of radioimmunoassay A commercially available digoxin kit (Schwarz/Mann) was used These determinations were performed 1–21 days before *exitus* In no case were changes in the maintenance therapy undertaken

Calculations

Conventional statistical methods were used Statistical significance of the difference between two sample means was evaluated by Student s *t* test The same distribution was used to test whether the correlation coefficient differed from zero

RESULTS

Tables 3 and 4 give the digoxin content (ng/g tissue wet weight) in the various tissues from adults and infants Both age groups show the same relative distribution of the glycoside (Fig 1) The highest digoxin concentrations were found in the choroid plexus (infants 287 ng/g adults 221 ng/g mean values) Somewhat lower levels were demonstrated in ventricular (245 and 133 ng/g respectively) and atrial (165 and 65 ng/g respectively) myocardium kidney (167 and 128 ng/g respectively) and liver (82 and 72 ng/g respectively) Low concentrations of the glycoside were found in skeletal muscle (31 and 30 ng/g respectively) brain (30 and 32 ng/g respectively) and subcutaneous fat (10 ng/g analysed only in adults) The mean digoxin concentrations in choroid plexus kidney liver

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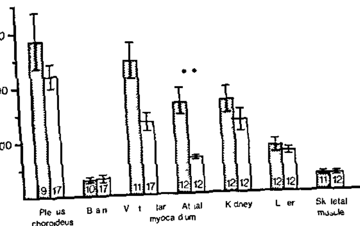


Fig 1 Digoxin content in various tissues (mean ±S E M) Stippled bars infants Unfilled bars adults Numbers within bars denote numbers of patients
* $p<0.01$ * $p<0.001$

where plasma concentrations of digoxin were determined during maintenance therapy at various time points before death the obtained values ranged between 0 9 and 1 9 ng/ml (mean 1 6 ng/ml) for adults and between 1 2 and 2 3 ng/ml (mean 1 7 ng/ml) for infants If a calculation is made using these plasma digoxin values the ratio between myocardial (ventricular tissue) and plasma concentrations varied from 47 1 to 174 1 (mean 104 1) in the adults and from 70 1 to 175 1 (mean 106 1) in the infants

DISCUSSION

The present analyses of the digoxin content in different tissues after maintenance therapy showed the same relative distribution of the glycoside in infants and adults choroid plexus>ventricular myocardium>kidney>liver >skeletal muscle An interesting finding was the difference in glycoside content within the heart the digoxin concentration in ventricular muscle being significantly higher than in atrial tissue A similar difference has been demonstrated in animal experiments Thus in dogs Deutscher et al (9) found the atrial concentration of digoxin to be only 45% of that in the left ventricle The reason for this difference is unclear but it might be due to differences in blood flow (9)

In infants the concentrations of the glycoside in kidney and in liver agreed well with those reported by Hernandez et al (14) using tritiated digoxin the concentrations in heart tissue on the other hand seemed to be somewhat higher The study by Hernandez et al (14) does not state whether the analyses were performed on atrial or ventricular tissue and this complicates a direct comparison with the present data Using radioimmunoassay Krasula et al (16) found an atrial digoxin concentration of 62±6 ng/g tissue in chronically digitalized children undergoing cardiac surgery this is significantly lower than the present value of 165±25 ng/g However the patients in the report of Krasula et al (16) were aged 1–12 years compared with 5 days to 8 months in the present study moreover their values are in the same range as those obtained for atrial tissue from adults in the present study 65±9 ng/g

Krasula et al (16) reported higher tissue concentrations of digoxin in acutely than in chronically digitalized children This agrees with the present finding that the two acutely digitalized neonates (Nos 1 and 2 Table 2) had a myocardial digoxin content higher than the

Table 3 *Tissue concentrations of digoxin (ng/g wet tissue mean±standard error of mean S E M) in adults*

Pat no	Choroid plexus	Brain	Ventricular myocardium	Atrial myocardium	Kidney	Liver	Skeletal muscle	Subcutaneous fat
1	242	30	111	27	85	39	25	15
2	233	4	87	78	74	36	19	23
3	143	74	76	50	56	46	19	15
4	72	3	167	37	83	89	56	17
5	373	68	161	72	189	45	35	9
6	224	17	169	81	253	131	55	5
7	235	5	131	51	71	186	36	4
8	313	41	224	112	213	64	71	8
9	209	60	50	27	54	29	31	11
10	340	21	296	129	154	86	25	7
11	145	45	121	46	113	61	26	4
12	205	55	211	73	186	49	13	4
13	228	3	80	–	–	–	–	–
14	408	37	62	–	–	–	–	–
15	105	34	84	–	–	–	–	–
16	145	11	91	–	–	–	–	–
17	141	41	144	–	–	–	–	–
Mean±S E M	221±23	32±6	133±16	65±9	128±20	72±13	30±4	10±2

skeletal muscle and brain did not differ significantly between infants and adults However significant differences were found between the glycoside concentrations in ventricular ($p<0.01$) and atrial ($p<0.001$) myocardium These differences are still significant if the two acutely digitalized neonates are excluded ($p<0.05$ and $p<0.001$ respectively) Within the heart there was a significantly higher concentration of digoxin in ventricular than in atrial tissue This was demonstrated both in infants ($p<0.05$) and in adults ($p<0.01$) Thus in the infants the digoxin concentration ratio between atrial and ventricular myocardium calculated from mean values was 2.3 In the adults the corresponding ratio was 1.2

There was a poor correlation between the myocardial (ventricle+atrium) and skeletal muscle content of digoxin Furthermore there seemed to be no direct relation between the tissue concentrations of the glycoside (myocardium skeletal muscle) and the daily maintenance dose expressed in mg/kg bw/24 h

In 7 adults (Table 1) and in 7 infants (Table 2)

Table 4 *Tissue concentrations of digoxin (ng/g wet tissue mean±standard error of mean S E M) in infants*

Pat no	Choroid plexus	Brain	Ventricular myocardium	Atrial myocardium	Kidney	Liver	Skeletal muscle
1	259	9	343	227	304	86	57
2	277	44	316	374	126	80	29
3	–	26	201	144	58	121	31
4	221	7	255	136	189	156	51
5	155	24	237	133	148	150	41
6	–	–	280	218	128	66	38
7	128	36	112	112	146	26	17
8	486	36	215	137	101	57	–
9	–	–	–	74	89	47	20
10	160	23	476	236	217	96	12
11	263	36	95	89	165	55	20
12	632	54	161	102	337	40	21
Mean±S E M	287±56	30±5	245±33	165±25	167±24	82±12	31±4

because of greater tissue stores in the infants than in the adults but the finding that most tissues in the body bind digoxin to the same extent in the two age groups does not favour this view To solve this problem further studies on digoxin pharmacokinetics in infants are needed

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REFERENCES

1 Akera T Baskin S J Tobin T & Brody T M Ouabain temporal relationship between the inotropic effect and the in vitro binding to and dissociation from (Na +K) activated ATPase *Naunyn Schmiedebergs Arch Pharmacol 277* 151 1973

2 Akera T Larsen F S & Brody T M Correlation of cardiac sodium and potassium activated triphosphatase activity with ouabaininduced inotropic stimulation *J Pharmacol Exp Ther 173* 145 1970

3 Aronsen R S Gelles J M & Hoffman B F Effect of ouabain on the current underlying spontaneous diastolic depolarization in cardiac Purkinje fibres *Nature New Biol 245* 118 1973

4 Auditore J V & Murray L Cardiac (microsomal) Na+K transport system *Arch Biochem Biophys* 99 372 1962

5 Bertler Å & Redfors A An improved method of estimating digoxin in human plasma. *Clin Pharmacol Ther 11* 665 1970

6 Bonting S L Simon K A & Hawkins N M Studies on sodium potassium activated adenosine triphosphatase I Quantitative distribution in several tissues of cat *Arch Biochem Biophys 95* 416 1961

7 Coltart D J Howard M R & Chamberlain D Myocardial and skeletal muscle concentrations of digoxin in patients on long term therapy *Br Med J II* 318 1972

8 Cree J E Coltart D J & Howard M R Plasma digoxin concentration in children with heart failure *Br Med J I* 443 1973

9 Deutscher R N Harrison D C & Goldman R H The relation between myocardial ³H-digoxin concentration and its hemodynamic effects. *Am J Cardiol* 9 47 197?

10 Doherty J E Perkins W H & Flanigan W J The distribution and concentration of tritiated digoxin in human tissues *Ann Int Med 66* 116 1967

11 Dungan W T Doherty J E Harvey C Char F & Dalrymple G V Tritiated Digoxin XVIII Studies in infants and children *Circulation 46* 983 197

12 Glynn J M The action of cardiac glycosides on ion movements *Pharmacol Rev 16* 381 1972

13 Hayes C J Butler Jr V P & Gersony W M Serum digoxin studies in infants and children *Pediatrics 52* 561 1973

14 Hernandez A Burton R M Pagtakhan R D & Goldring D Pharmacodynamics of H-digoxin in infants *Pediatrics 44* 418 1969

15 Iisalo E Dahl M & Sundquist H Serum digoxin in adults and children *Int J Clin Pharmacol 7* 219 1973

16 Krasula R. W Hastreiter A R Levitsky S Yanagi Ruth & Soyka L F Serum atrial and urinary digoxin levels during cardiopulmonary bypass in children *Circulation 49* 1047 1974

17 Krasula R. W Pellegrino P A Hastreiter A R & Soyka L F Serum levels of digoxin in infants and children *J Pediatr 81* 566 1972

18 Krasula R W Yanagi Ruth Hastreiter A R Levitsky S & Soyka L F Digoxin intoxication in infants and children Correlation with serum levels *J Pediatr 84* 265 1974

19 Lee K S & Klaus W The subcellular basis for the mechanism of inotropic action of cardiac glycosides *Pharmacol Rev 23* 193 1971

20 Lullman H & van Zwieten P A The kinetic behaviour of cardiac glycosides in vivo measured by isotope techniques *J Pharm Pharmacol 21* 1 1969

21 Miller W W & Gilliland K Developmental differences in canine myocardial adenosine triphosphatase activity *Circulation 66–67* suppl IV abs No 140 1972

22 Neill Catherine A The use of digitalis in infants and children *Progr Cardiovascular Dis 7* 399 1965

23 Okita G T Relationship between Na K ATPase inhibition and the inotropic and cardiotoxic actions of digitalis *Symposium on digitalis Oslo* Norway (ed O Storstein S Nitter Hauge & L Storstein) Gyldendal Norsk Forlag Oslo Norway 1973

24 Okita G T Richardson F & Roth Schechter F F Dissociation of the positive inotropic action of digitalis from inhibition of sodium and potassium-activated adenosine triphosphatase *J Pharmacol Exp Ther 185* 1 1973

25 O Malley E. N Coleman W B & Stevenson I H Plasma digoxin levels in infants *Arch Dis Child 48* 55 1973

26 Portius J J & Repke K Die Wirkung von Herzglykosiden auf verschiedene ATPase des Herzmuskels in Abhängigkeit von Ionen milien *Naunyn Schmiedebergs Arch Pharmakol 243* 335 1962

27 Redfors A Bertler Å & Schuller H The ratio between myocardial and plasma levels of digoxin in man *Symposium on digitalis Oslo* (ed O Storstein S Nitter Hauge and L Storstein) Gyldendal Norsk Forlag Oslo 1973

28 Repke K Effect of digitalis on membrane adenosine triphosphatase of cardiac muscle Drugs and Enzymes *Proceedings of 2nd International Pharmacological Meeting* Pergamon Press New York 1965

29 Rogers M C Willerson J T Goldblatt A & Smith T W Serum digoxin concentrations in the human

mean In adults the digoxin concentrations in the investigated tissues are in the same range as those reported by other investigators (e g 7 10 27)

In the present study, tissue digoxin values are given in ng digoxin/g tissue, wet weight Doherty et al (10) analysed tissue digoxin concentrations in adult patients after administration of a single dose of tritiated glycoside No correlation was found between patients with varying degrees of congestive heart failure and the dry/wet tissue ratio We have therefore assumed that also the normal variation with age in the ratio intracellular/extracellular fluid is of minor importance in relation to the distribution of digoxin

Plasma digoxin concentrations during maintenance therapy could not be determined in all the present patients However based on the available values and on previous results with the digoxin doses used in the different age groups there could be expected in infants and adults the same plasma concentration or a slightly higher one in infants (32) No simple relationship between plasma and myocardial concentrations of digoxin seems to exist (7 27) Thus it is unlikely that the demonstrated difference in digoxin content in the myocardium from infants and from adults is attributable to differences in plasma concentrations only Furthermore the digoxin levels in kidney liver skeletal muscle and brain did not differ significantly in the two groups Therefore the present results suggest a higher degree of binding of digoxin to the myocardium of the infants

Cardiac glycosides are known to bind specifically to and to inhibit the sarcolemmal transport enzyme Na^+–K^+ activated ATPase (12) Tissues shwoing a high activity of this enzyme might therefore to a large extent be expected to bind digoxin The digoxin content in the choroid plexus a tissue known to have a high activity of Na^+–K^+ ATPase (6) was the highest among the tissues investigated in the present study thus supporting such a view Brain tissue, on the other hand is also known to contain large amounts of Na^+–K^+ ATPase (6), but only low concentrations of digoxin probably owing to the presence of a blood-brain barrier for the glycoside (10) A low digoxin concentration in brain tissue was also found in the present study

It has been suggested that the inotropic effect of cardiac glycosides is the result of inhibition of the Na^+–K^+ ATPase in the sarcolemmal membrane (1 2 4, 26, 28) However this has been questioned as it was possible to dissociate the inotropic effect and the inhibition of Na^+–K^+ ATPase produced by cardiac glycosides (23 24) On the other hand inhibition of this enzyme seems to be responsible for the electrophysiological and toxic effects of the glycosides (3 19 31)

Miller & Gilliland (21) have shown that the median myocardial Na^+–K^+ ATPase activity was significantly greater in foetal and newborn puppies than in mature dogs If this is valid for humans it might be speculated that more digoxin is needed in infants than in adults to inhibit this enzyme (30) This might explain why infants tolerate higher doses of digoxin per unit weight and why signs of toxicity manifest at higher plasma concentrations in infants than in adults It might also explain the present findings of a higher myocardial digoxin content in infants than in adults However it has been suggested that only a small fraction of the digoxin in heart muscle is bound to active receptor sites (20) and it is not known whether this fraction is in equilibrium with the greater amounts unspecifically bound to the tissues Therefore the question to what extent an increased total tissue concentration of digoxin will reflect increased binding to Na^+–K^+ ATPase remains to be solved

A higher activity of myocardial Na^+–K^+ ATPase might thus explain why children tolerate plasma concentrations of digoxin that are definitely toxic in adults However differences in Na^+–K^+ ATPase activity cannot explain why infants need higher doses of digoxin than adults to reach the same plasma concentrations (32) Theoretically this can be attributed to a greater apparent volume of distribution

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A 21 YEAR PSYCHO SOCIAL FOLLOW UP OF 524 UNSELECTED CASES OF DOWN S SYNDROME AND THEIR FAMILIES

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ABSTRACT Øster J and van den Tempel A (Department of Paediatrics Centralsygehuset Randers Denmark) A 21 year psycho-social follow up of 524 unselected cases of Down s syndrome and their families. Acta Paediatr Scand 64 505 1975 —The death rate was followed and a life table has been constructed Three crucial events were examined 1 the period of diagnosis 2 the problem of institutionalization versus homecare 3 the ageing and death of the parents and its consequences for the person with D.S Several other medical problems psychological implications and social consequences for the person with D S for his parents and siblings are highlighted The worst problem for parents with the child at home was social isolation for the parents with the child at an institution it was the very existence of the child To both groups the most encouraging fact was that their child was happy friendly and in good spirits Finally emphasis is laid upon the medical and paramedical problems which must be discussed and solved to make a harmonious and social life for the person with D S for his normal siblings and for the parents

KEY WORDS Down s syndrome family problems social consequences psychological implications

Longitudinal studies on large unselected materials concerning psycho social problems in families with a child with Down s syndrome (D S) are non existent although such prospective studies are in preparation Furthermore there are only a few comprehensive studies which consider these problems at all namely those by Kramm (2) Chigier (1) and Lavoie (3) The first one is concerned with 50 families who had their D S child at home the next one comprises 118 families with the child at home and 66 institutionalized the last one consists of 109 families but has not been published as yet All three studies were done by personal interview technique

Several studies exist concerning special or partial problems in the field of sociology psychology education and the family situation but this is rather modest in quantity in view of the extent and gravity of the condition Since 1959 it seems as if scientific interest has mostly been concerned with cytogenetic and biochemical studies

The present work is a follow up study of patients with Down s syndrome and their families The material was collected in 1949 (4) 524 cases of Down s syndrome were registered from a well defined part of Denmark comprising all cases living in that area in 1949 The study was a clinical and genealogical investigation the final conclusion being that Down s syndrome did not seem to be a hereditary disease

The discovery by Lejeune and others from 1959–60 showed that Down s syndrome *is* a genetically determined disease the cause of which is still obscure The discovery was epoch making concerning etiology prevention

fetus neonate and infant *N Engl J Med* 20 1010 1972

30 Rutkowski Monika M Cohen S N & Doyle E F Drug therapy of heart disease in pediatric patients II The treatment of congestive heart failure in infants and children with digitalis preparation *Am Heart J* 86 270 1973

31 Ten Eick R E Bassett A L & Okita G T Dissociation of electrophysiological and inotropic actions of strophanthidin 3 bromo acetate possible role of adenosine triphosphatase in the maintenance of the myocardial transmembrane Na and K gradients *J Pharmacol Exp Ther* 185 12 1973

32 Wettrell G Andersson K E Bertler Å & Lundström N R Concentrations of digoxin in plasma and urine in neonates infants and children with heart disease *Acta Paediatr Scand* in press

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Table 1 *Parental attitude towards future children in 27 families with the child at home (Home Gr) and 32 families with the child at an institution (Inst Gr)*

	Home Gr	Inst Gr
No desire	14	19
Afraid of defects	4	5
Afraid of burden	2	2
Active desire	7	6
Had more children	10	8

Many parents were struck by the doctor s doubtfulness and hesitation concerning the nature of the condition and its consequences for the social and educational development of child and family They often felt they ran up against a wall of secrecy and ignorance or they felt an evasive reaction which left them in a state of perplexity

At best they were referred to other physicians most often pediatricians or to pediatric hospitals And here they most often were only given advice to send the child to an institution which did not give much support to the intellectual or emotional needs of the parents Of 32 families who sent the child to an institution 24 did not feel that they had got any advice at all while only 8 out of 27 who kept the child at home felt in the same way More than half of the parents (58%) who were told the diagnosis felt that they had no opportunity to discuss the future consequences and only half of the parents were invited to discuss the problem of home versus institution But practically all parents stated that they had a reasonable amount of time to think for themselves before they made the final decision Nobody had any contact with or was referred to social workers or educators

Only one single family received advice to have more children As a matter of fact only a minority of the parents wanted more children they were rather old they feared a repetition or they feared the burden of the patient plus another baby (Table 1) Some of them had an active desire for more children and several of them indeed had more children Most often the parents agreed concerning future children

Home versus institution

The problem of home versus institution for a child with Down s syndrome is another great and important problem

The main reason for sending the child to an institution was exhaustion of the mother—the excess work load created by the child s condition was so intense that the mother s strength did not suffice (Table 2) Several institutionalizations were made because one or both of the parents died Furthermore many parents emphasized that the retarded child disturbed the development of the siblings and their normal existence Several parents found it best for the retarded child to be among equals And in many cases it was the medical advice given by physicians whom the family trusted which was the main motive in the institutionalization No doubt the authority of the medical profession and the fact that the parents did not have advice from other professionals e g social workers or educators are co responsible factors in many cases for this attitude Economic or housing problems only rarely had any importance

Most of the children were between 5 and 1[illegible] years old when they left home probably because the children at that age very often are restless must often be under constant supervision may have a tendency to run away from home etc But there are also parents who fel that the abnormal child was better cared for and managed at an institution than at home with it open community reflections on the futur occurred very early to many parents an

Table 2 *Reasons for referring the child to a institution in 227 out of 366 families*

Mother exhausted	80
Broken family by death	47
Disturbances of brothers and sisters	44
Physician s advice	43
Best for the patient	36
Other reasons	26
No answer	29

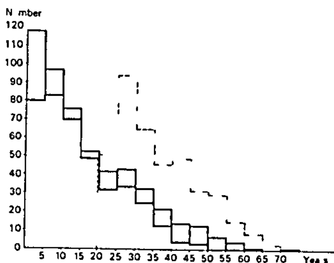


Fig 1 Age distribution of 524 cases of Down s syndrome 1949 (—) and of 395 living at the follow up 1971 ( ) 129 died 1949–1971

and so on But living persons with Down s syndrome and their families and their lives were on the whole uninfluenced by these facts The aim of the present work is to elucidate some aspects of the existing problem What does it mean to have a person with Down s syndrome in the family whether he is at home or in an institution? How does this fact influence the lives of parents and siblings?

MATERIAL AND METHODS

The forementioned 524 cases have been followed up three times in 1959 and 1971 by correspondence and in 1972 by intensive personal interviews with selected families Due to the small size of the country and the very limited migration rate of the Danish population it has been possible to ascertain the lives of everyone

At the last follow up 395 were alive Through correspondence it was possible to contact 366 families who gave information and answered questionnaires concerning the lives of their children with Down s syndrome and the lives of the parents and their healthy children In 6 other cases the given information was useless 23 families did not respond to the inquiries they wanted to be left in peace—an attitude which must be respected

But the majority were eager to answer the questions and often gave supplementary information and statements considering that this might prove helpful to other parents who in the future had to face the same problems as they had experienced

Furthermore a selection was made according to social criteria of the families and 27 families with their child living at home and 32 families with their child institutionalized were chosen for personal interviews to elucidate the psycho social factors which may be involved with the existence of the patient

Thus there were two groups First the total material consisting of *366 families* followed by correspondence second the *59 families* (27+32) who were further investigated by intensive interviews

All these psycho social studies cannot be treated in an ordinary scientific statistical way The material is limited although intensively examined Control material does not exist It is abundant in feelings statements case records and personal convictions The truth of it may however be as significant as any other experience—and it can be a leitmotif a help and a consolation for future parents

RESULTS

Survival and death

In 1959 73 had died, and in 1971 another 56 In Fig 1 are found the age distribution of the original material and the 129 persons who died between 1949 and 1971 There are many more in the older age groups i e ≥30 or 50 years of age (250 rsp 57) than 20 years ago (95 rsp 13)

The 129 dead persons primarily originated from the age group 0–4 years In all age groups and in both periods patients with Down s syndrome have a considerable excess mortality most pronounced in the age group 0–4 years The excess mortality in the age group ≥10 years decreased in the last period not much but significantly At the same time the tendency to an excess mortality for patients in institutions in period 1 disappeared in period 2

The patients in the two succeeding periods died from the same illnesses but it is evident that the excess mortality from infections and respiratory diseases decreased—from 52 to 12 respectively from 124 to 62 from the first to the second period These problems are described elsewhere (5)

Early diagnosis

Most mothers had a suspicion at birth or within a few days that something was wrong with the baby The medical diagnosis in most cases was postponed a while but was given within the first year in the majority of cases Most parents felt that the physician should give the diagnosis as soon as he is convinced of its reliability but not on first suspicion

Table 7 *Diseases among 366 persons with Down s syndrome*

	Home Gr	Inst Gr	Total
Infections	7	4	11
Epilepsy	4	5	9
Impaired sight/hearing	1	12	13
Obesity	3	0	3
Senile decay	4	6	10
Diabetes	1	1	2

only tolerable or poor In childhood the tendency to infections persists Epilepsy is about 3 times more frequent than in the general population The patient is often sensorially de prived by impaired sight and hearing it is no wonder that 10 out of 366 are characterized by senile decay as there is a tendency toward early senility Only 3 from the home group suffered from obesity

The intensive interviews gave some further details Many parents felt that their children suffered from speech defects with indistinct speech as the major complaint and with no speech at all in 15 out of 32 patients in the institution group they received little or no treatment neither instructions nor guidance about how to deal with the problem The parents felt that the communication problems of these retarded children were greater than they should have been because proper treatment or guidance had not been given

The parents of the home group referred to their children with Down s syndrome as lively and active only a single one apathetic In most cases they could be left alone at home (they were all more than 20 years old) But in some cases mothers did not seem to trust them enough to leave them alone in nine out of 27 cases this prevented the mother from taking work outside the home Some were described as boisterous when they got into trouble Practically all managed their everyday problems such as clothing toilet eating Nearly everybody had an active social attitude being present when there were guests and participating in discussions For most of them holidays were arranged often by and with the parents but most often by the Mental Retardation Service this was appreciated alike by the patient and the parents

The parents were asked whom they would apply to in case of problems in the family or with their retarded child again Mental Retardation Service would be consulted

About half (14 out of 27) of the home group were occupied in some sheltered workshop Most of the remaining had been offered the same but refused for many reasons too many clients at the workshop too fast a speed too routine an occupation etc Most of these reasons are more excuses for not sending the child than real facts

It is striking that 20 out of 27 never attended any school outside the home which is felt as a great lack and a need which must be met in the future Only 4 (out of 27) went to school 4–12 years Most parents felt that society had not done enough to help them keep the child at home apart from the fact that all 27 now get a pension

Eight parents in the institution group felt that society might have furnished the family with some help which might have prevented them from sending their child to an institution namely possibilities for day-care (2) for home help (2) for school or workshop (2) and for more economic support (1)

On the other hand the parents view on a multidisciplinary clinic for handicapped children was divided although the majority (33 out of 59) had a positive attitude and emphasized that it would be an advantage because (*a*) more specialists could be consulted (*b*) they could get more diverse information and instructions (*c*) they might avoid a stereotyped medical pattern

Mother s health and family problems

At the interviews the health of the mothers was investigated in details from an emotional and a physical point of view Although we have no controls it was the general impression that there was an even distribution of disturbances

Table 3. *Reasons for keeping the child at home in 139 out of 366 families*

Could not do without the patient	52
Best for the patient	45
The service too bad	11
Advice from service/physician	10
Other reasons	4
No answer	24

Table 5. *Survey of parents' agreement or disagreement concerning home versus institution*

	Home Gr.	Inst. Gr.	Total
Agreed	65	130	195
Disagreed	1	12	13
No information	73	85	158
Total	139	227	366

persuaded them that they would prefer to see their child in an institution, happy if possible, before they die themselves. But nobody could tell us the optimal time for such an institutionalization.

The parents who kept their children at home had many positive and good reasons for doing so (Table 3). For the majority it was due to emotional feelings and a conviction that the development of the child would profit from it; here they are in accordance with recent scientific findings.

Most of the parents were convinced that the decision they made, whether home or institution, was a good one (Table 4). This especially holds true for the parents who kept their child at home. In the group which sent the child away several parents felt the decision was questionable or wrong.

In 1949 only 47 per cent of the patients were in institutions; the figure for 1959 was 58—and for 1971, 63 per cent.

The number of living males (197) and females (198) were the same, but more men (130) than women (117) were institutionalized.

Generally the parents agreed upon this important decision of homer versus institution (Table 5). But several parents now admit, many years afterwards, that actually they disagreed; most often it was the father who wanted the child sent to an institution, against the wish of the mother, but the reverse was found in a single case.

We asked how the family felt the decision influenced the parents, the siblings and the child. Table 6 shows a tendency to consider it good for the child to be institutionalized and good for the brothers and sisters, several of which had been badly affected by his staying at home. Only few considered it good for the siblings to keep the patient at home.

Table 4. *Parents' opinion concerning the decision of home versus institution*

	Home Gr.	Inst. Gr.	Total
Good	103	159	262
Bad	3	5	8
Both/and	6	18	24
No answer	27	45	72
Total	139	227	366

Condition of the person with Down's syndrome

The health of the patients was generally considered to be good without any significant difference between the home and institution groups. In 78 cases health was described as

Table 6. *The effect of the decision on parents, brothers, sisters and child—as interpreted by near relatives*

	Home Gr.	Inst. Gr.	Total
Parents			
Good	45	50	95
Neutral	43	44	87
Bad	6	4	10
No information	45	129	174
Brothers and sisters			
Good	35	56	91
Neutral	58	51	109
Bad	9	1	10
No information	37	119	158
Patient			
Good	65	105	170
Neutral	27	22	49
Bad	1	5	6
No information	46	95	141

Table 9 *Survey of the family s contact with the service for the mentally retarded in the home and institution group*

	Home Gr	Inst Gr
Never	6	27
Infrequently	8	5
Once a year	10	0
Regularly	3	0
Total	27	32

and discuss the whole problem with experts and thus get skilled advice

Parents with retarded child in an institution

For the parents in the institution group the largest single problem was that the existence of the child although he was away from home put pressure on them The majority (165) of these 237 parents had some or much contact with their child and the institution But there remain 46 cases i e 20% with little or no contact at all And several of these parents seemed to suffer from this In only 16 cases was there no information on this point

Contact with the Service

The home and institution groups have been compared concerning contact with the Mental Retardation Service (Table 9) Apart from the institution and its personnel families in the institution group did not seem to make use of communication with the Service whereas most of the parents in the home group used the consultations of the Service But about half of the parents considered the visit by social workers as unimportant and trivial they had lived so long with the problem and had their own experience several problems had been solved and others had been repressed so could not be rediscussed with professionals who had only a superficial knowledge of the family

Parents interest in knowledge and joining groups

Several parents tried hard to learn something about mental retardation and Down s syndrome in particular by reading and through the Parents Associations which have existed in Denmark since 1951 These associations and in recent years mass media communication helped the parents face the truth accept the realities and yet make a good life for their retarded child for the healthy siblings and for themselves Of the group with an institutionalized child there was more parents than in the home group who had done nothing to increase their knowledge and understanding concerning their child s condition

But only less than half of all parents were members of the Parents Association (Table 10) The parents of the institution group seemed to be especially reserved although this group often seemed to be in need of much emotional support

DISCUSSION

Parents who sent their D S child to an institution

When the diagnosis and its consequences were presented to these parents they felt very uncertain as to their family role They realized this situation required great skill and effort and they felt inadequate to deal with the problem of how to handle the child with D S in the best way without damaging the other children

Furthermore they were only offered counselling and guidance by their physician who most often from tradition urged them to send the child to an institution This advice was often in conflict with the innermost feelings and convictions of the parents particularly the mother On the other hand the parents were aware that

Table 10 *Survey concerning parents membership of the Parents Association*

	Home Gr	Inst Gr	Total
Yes	65 (47 %)	90 (40 %)	155
No	34 (24 %)	93 (41 %)	127
No information	40	44	84
Total	139	227	366

Table 8 *Parents situation (alive or dead) in 366 cases of Down s syndrome*

	Home Gr	Inst Gr	Total
Both parents alive	82	92	174
Mother alive	38	38	76
Father alive	5	17	22
Both parents dead	14	80	94
Total	139	227	366

in mothers of both groups and that the placement of the child in an institution did not influence the health of the mothers

The mothers were on average 34 years old at the birth of the patient His presence in most cases did not influence marital relations In some cases the influence seemed to have been positive The stability of the marriages seems to have been good among 372 families whose marriage was known divorce existed in only 25 cases 20 children of these families were in an institution and only 5 remained in the mother s home In a few cases the condition of the child was contributory to the divorce In 8 cases it *is* established that this was not the case In another 12 cases the matter is unsettled

The incidence of divorce about 7% is lower than in the general population of the same age which may be due to the fact that this special baby called for solidarity

Through interviews with the parents of the home group it was furthermore confirmed that the retarded child rather often was considered to have restrained the social contact and activities of brothers and sisters Some mothers felt they neglected their healthy children in favour of the sick child

The greatest single problem for the parents of the home group is that they have been restricted in their activities while most of them did not consider that the mother s leisure time had been reduced and stated it without regret In only 7 out of 27 cases the child s condition limited the mother s leisure time much

Most of the home patients were regularly taken with the mother for shopping in most cases the father shared the responsibility for this child with the mother In this respect he now replaced the siblings who all had left the home

In the course of the interviews it became clear that several parents were ashamed of the retarded child or were unpleasantly affected by the tactlessness of others Although several parents stated that they did not mind other people s opinions, most of them admitted that this attitude might be a passive defensive attitude not wanting to dignify the criticism or thoughtlessness of others

The parents of both home and institution groups agreed that the most encouraging fact about their children was that they were happy friendly, and most often good humoured

Changing family structure over time

In the course of time the family structure also changed in other respects the brothers and sisters grew older they had children they died (in 66 families out of 372 well known) Practically all (395) mothers had gone through menopause so that the families were complete In 16 of 372 well known families there are additional cases of Down s syndrome With advancing age of the patients the parents also grow older and die In 1971 only 174 had both parents (Table 8) and it seemed to be the presence of the mother which kept the patient at home When she or both parents died the children were most often transferred to an institution In 14 cases where they were kept at home in spite of both parents death they were in a sister s house in 9 and in a brother s house in only 3 cases

What is going to happen to the patient with D S when the parents die? Most parents stated that he should be sent to an institution (22) and only a minority (2) made their healthy children promise that they would take care of him 3 were undecided Probably this point is the one which most worries the parents as they grow older They might be greatly relieved if they were to visit an institution observe the life in it

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Table 8 *Parents situation (alive or dead) in 366 cases of Down s syndrome*

	Home Gr	Inst Gr	Total
Both parents alive	82	92	174
Mother alive	38	38	76
Father alive	5	17	22
Both parents dead	14	80	94
Total	139	227	366

in mothers of both groups and that the placement of the child in an institution did not influence the health of the mothers

The mothers were on average 34 years old at the birth of the patient His presence in most cases did not influence marital relations In some cases the influence seemed to have been positive The stability of the marriages seems to have been good among 372 families whose marriage was known divorce existed in only 25 cases 20 children of these families were in an institution and only 5 remained in the mother s home In a few cases the condition of the child was contributory to the divorce In 8 cases it *is* established that this was not the case In another 12 cases the matter is unsettled

The incidence of divorce about 7% is lower than in the general population of the same age which may be due to the fact that this special baby called for solidarity

Through interviews with the parents of the home group it was furthermore confirmed that the retarded child rather often was considered to have restrained the social contact and activities of brothers and sisters Some mothers felt they neglected their healthy children in favour of the sick child

The greatest single problem for the parents of the home group is that they have been restricted in their activities while most of them did not consider that the mother s leisure time had been reduced and stated it without regret In only 7 out of 27 cases the child s condition limited the mother s leisure time much

Most of the home patients were regularly taken with the mother for shopping in most cases the father shared the responsibility for this child with the mother In this respect he now replaced the siblings who all had left the home

In the course of the interviews it became clear that several parents were ashamed of the retarded child or were unpleasantly affected by the tactlessness of others Although several parents stated that they did not mind other people s opinions, most of them admitted that this attitude might be a passive-defensive attitude, not wanting to dignify the criticism or thoughtlessness of others

The parents of both home and institution groups agreed that the most encouraging fact about their children was that they were happy friendly and most often good humoured

Changing family structure over time

In the course of time the family structure also changed in other respects the brothers and sisters grew older they had children they died (in 66 families out of 372 well known) Practically all (395) mothers had gone through menopause so that the families were complete In 16 of 372 well known families there are additional cases of Down s syndrome With advancing age of the patients the parents also grow older and die In 1971 only 174 had both parents (Table 8) and it seemed to be the presence of the mother which kept the patient at home When she or both parents died the children were most often transferred to an institution In 14 cases where they were kept at home in spite of both parents death they were in a sister s house in 9 and in a brother s house in only 3 cases

What is going to happen to the patient with D S when the parents die? Most parents stated that he should be sent to an institution (22) and only a minority (2) made their healthy children promise that they would take care of him 3 were undecided Probably this point is the one which most worries the parents as they grow older They might be greatly relieved if they were to visit an institution observe the life in it

Table 9 *Survey of the family s contact with the service for the mentally retarded in the home and institution group*

	Home Gr	Inst Gr
Never	6	27
Infrequently	8	5
Once a year	10	0
Regularly	3	0
Total	27	3[illegible]

and discuss the whole problem with experts and thus get skilled advice

Parents with retarded child in an institution

For the parents in the institution group the largest single problem was that the existence of the child although he was away from home put pressure on them The majority (165) of these 237 parents had some or much contact with their child and the institution But there remain 46 cases i e 20% with little or no contact at all And several of these parents seemed to suffer from this In only 16 cases was there no information on this point

Contact with the Service

The home and institution groups have been compared concerning contact with the Mental Retardation Service (Table 9) Apart from the institution and its personnel families in the institution group did not seem to make use of communication with the Service whereas most of the parents in the home group used the consultations of the Service But about half of the parents considered the visit by social workers as unimportant and trivial they had lived so long with the problem and had their own experience several problems had been solved and others had been repressed so could not be rediscussed with professionals who had only a superficial knowledge of the family

Parents interest in knowledge and joining groups

Several parents tried hard to learn something about mental retardation and Down s syndrome in particular by reading and through the Parents Associations which have existed in Denmark since 1951 These associations and in recent years mass media communication helped the parents face the truth accept the realities and yet make a good life for their retarded child for the healthy siblings and for themselves Of the group with an institutionalized child there was more parents than in the home group who had done nothing to increase their knowledge and understanding concerning their child s condition

But only less than half of all parents were members of the Parents Association (Table 10) The parents of the institution group seemed to be especially reserved although this group often seemed to be in need of much emotional support

DISCUSSION

Parents who sent their D S child to an institution

When the diagnosis and its consequences were presented to these parents they felt very uncertain as to their family role They realized this situation required great skill and effort and they felt inadequate to deal with the problem of how to handle the child with D S in the best way without damaging the other children

Furthermore they were only offered counselling and guidance by their physician who most often from tradition urged them to send the child to an institution This advice was often in conflict with the innermost feelings and convictions of the parents particularly the mother On the other hand the parents were aware that

Table 10 *Survey concerning parents membership of the Parents Association*

	Home Gr	Inst Gr	Total
Yes	65 (47%)	90 (40%)	155
No	34 (4%)	93 (41%)	127
No information	40	44	84
Total	139	2?7	366

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of Health Education and Welfare Washington D C 1963
3 Lavoie B B An ecologic study of families with Down s syndrome children in the Washington metropolitan area Diss Washington D C 1974
4 Øster J Mongolism A clinico-genealogical investigation comprising 526 mongols living on Seeland and neighbouring islands in Denmark Danish Science Press Copenhagen 1953
5 Øster J Mikkelsen M & Nielsen A Mortality and life table in Down s syndrome *Acta Paediatr Scand* 64 32? 1975

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institutionalization would relieve their role as parents to that special child and so enable them to fulfil their function as parents to the healthy siblings in an optimal way

As they most often did not get any help from educators, psychologists or social and community workers they became exhausted by heavy physical and emotional strain so that they began to doubt their ability to develop the sick child s capabilities and personality

Parents were afraid that they would have inadequate relationships with their normal children and that the identification process would suffer The Down s syndrome child became an obstacle in the family hindering the ordinary socialisation process of the child no member of the family could play his role adequately The only solution was to place the Down s syndrome child in an institution where he could get the special developmental help the family was unable to give Finally they had no other choice than to transfer the child to an institution But it was a final resort and while the social structure of the family had changed physical removal did not remove the emotional effects From a sociological point of view this child is circling outside the family structure because the institution had taken over the responsibility of substituting the normal family

Parents with the D S child at home

The parents who kept their D S child at home took a fundamentally different attitude towards that special child and the problems involved They anticipated that they were able to intensify their role as parents although it might reduce their contact with others leading to a certain degree of social isolation They felt that this child with its biologically determined limitation would be able to develop its potential capacities best within the total family structure At the same time they realistically realized the reduced possibilities for the child s development These parents were convinced of the importance of good overall family relations in order to promote and realize the process of socialisation for every member of the family group But it cost these parents great emotional and physical effort to deal with these problems also because they, too had no other help than medical advice Not only did they become socially isolated the mothers specially were physically emotionally strained by lack of practical relief in everyday life and by their doubts whether they had acted wisely and correctly towards the child and the healthy siblings or not Finally the great work and the strong personal binding to the sick child rather often resulted in overprotection and dependency on others on behalf of the D S child

Conclusion

The child with D S and its biologically determined limitations cause a very complicated set of problems for the child his future and his family The basis and precondition for an adequate or normal function of that child and his family is among other things that the following problems are discussed with the family and brought to a solution

1 What does it mean to these parents and siblings to have this abnormal child whether he is at home or in an institution?

2 Do the parents have any possibility of handling the problems and reacting in an adequate way?

3 How are the parents able to fulfil their role as parents in an optimal way?

4 When and how shall the parents cope with the different processes of education and training of the D S child?

5 How can the parents solve the problems of identification and socialisation in an optimal way?

6 How do the parents estimate the aims and goals of the lives of their D S child their normal children and themselves?

REFERENCES

1 Chigier E *Mongolism in Tel Aviv A medico-social cross cultural study* Research Project B SS-CD-IS-6 Medical Ecology Dept Hebrew University Medical School Jerusalem 1969

2 Kramm E R *Families of mongoloid children* Children s Bureau Publication Number 401 US Dept

Table 1 *Clinical data*

Patient no	Sex	Length of gestation (weeks)	Birth weight (g)	Diagnosis	Long term phenobarbital treatment instituted at the age of
1	M	40	2 700	Hypoglycemia small for date convulsions	2 days
2	M	42	4 350	Intracranial bleeding convulsions	2 days
3	F	39	3 530	Herpes simplex encephalitis suspected seizures Ad mortem at 3 weeks of age	
4	F	39	2 670	*Severe birth* asphyxia, convulsions Ad mortem at 4 months of age	9 days
5	M	36	1 660	Prematurity moderate birth asphyxia chondrodystrophia calcificans convulsions	
6	M	40	4 110	Convulsions of unknown origin	11 days
7	M	39	3 035	Moderate birth asphyxia convulsions	
8	M	41	3 100	Moderate birth asphyxia convulsions	2 weeks
9	M	39	3 100	*Moderate birth* asphyxia convulsions	7 days
10	M	40	2 680	Severe birth asphyxia small for date suspected seizures Ad mortem at 3 weeks of age	
11	M	40	3 610	Convulsions of unknown origin	6 days
12	M	40	3 260	Hypoglycemia intravasal *coagulation convulsions*	
13	M	40	3 930	Severe birth asphyxia (breech presentation) suspected seizures	
14	M	41	3 680	*Severe* birth asphyxia (breech presentation) bronchopneumonia suspected seizures. Ad mortem at 8 days of age	
15	M	41	4 050	Moderate asphyxia intracranial bleeding convulsions	
16	M	39	3 640	Rh immunisation *moderate birth asphyxia convulsions*	
17	M	36	2 210	Prematurity birth asphyxia IRDS convulsions Ad mortem at 5 days of age	
18	M	43	2 710	Small for date suspected septicemia convulsions	3 weeks

MATERIAL AND METHODS

Patients

The study includes 18 infant patients subjected to anticonvulsive treatment during the neonatal period Most infants with repeated convulsions in our neonatal ward during a 2½-year period have been studied Clinical data are given in Table 1

The routine investigation schedule has included determinations of the blood concentrations of glucose calcium magnesium and sodium as well as an analysis of amino acids in urine and blood Lumbar puncture has been performed in most cases for microscopic spectrophotometric and bacteriologic/virologic examination When possible EEG has been recorded before the administration of phenobarbital and during the EEG recording 100 mg pyridoxine has been given intravenously

Throughout the investigation diazepam was given by injection to infants with frequent or prolonged convulsions considered to need an anti-convulsive drug with immediate effect In cases where the combination of diazepam and phenobarbital did not have sufficient anti-convulsive effect lidocain was given by infusion as described by Norell & Gamstorp (15)

The cases in which long term phenobarbital treatment has been instituted are listed in Table 1 The indications for institution of such treatment have not been strictly fixed during the investigation the major criteria have been recurrence of convulsions and epileptogenic EEG activity

Drug

Phenobarbital (Fenemal ACO Co) has been given as 15 mg tablets or as a solution for injection containing 20 mg phenobarbital per ml (a dilution of the commercial preparation) The tablets contain phenobarbital as the free acid In the solution (pH 8 5–9 5) only about 10% is free acid and the rest is sodium phenobarbital The tablets were pulverized before administration and given in milk with a spoon or by gavage

Dosage

Patients 1 and 2 were given daily oral doses approximately 7 5 mg/kg/day according to the current routine in the early part of the study In the course of the investigation the first (and often single) dose was then gradually increased as can be seen in Table 2 When the doctor in charge of the neonatal ward found indications for phenobarbital treatment the dose and way of administration was decided on in cooperation with the author In patients 3 to 18 repeated acute treatment doses were given only if the child had new convulsions after the first administration of phenobarbital and never earlier than 24 hours after the previous dose

Sampling

Capillary blood was sampled in heparinized tubes routinely used for hematocrit determinations The first sample from each patient was always taken immediately before the first administration of the drug As a rule blood

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PLASMA CONCENTRATIONS OF PHENOBARBITAL IN THE TREATMENT OF SEIZURES IN NEWBORNS

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ABSTRACT Jalling, B (Departments of Paediatrics and Clinical Pharmacology Karolinska Hospital, Stockholm, Sweden) Plasma concentrations of phenobarbital in the treatment of seizures in newborns. *Acta Paediatr Scand 64 514, 1975*—*The plasma concentration of* phenobarbital given as anticonvulsive treatment in the newborn period has been followed in 18 infants With constant daily doses the drug accumulated for at least 5 days After intramuscular injection of a single dose 90% of the peak concentration was reached within 4 hours in 8 of the 10 infants. The peak concentration (in μg/ml) approximately equalled 1.3×the dose (in mg/kg) Absorption after oral administration was less reliable In 12 of the infants the clinical course allowed attempts to evaluate the anticonvulsive effect of phenobarbital In 4 cases the convulsions continued In those 8 infants where phenobarbital seemed to be effective the approximate range of phenobarbital concentration when convulsions ceased was 12–30 μg/ml Phenobarbital half life ranged between 59 and 182 hours In some infants the rate of phenobarbital disappearance from the plasma varied considerably from day to day The pathological conditions causing seizures probably influence the distribution metabolism and excretion of the drug For the often seriously ill infants with convulsions it is therefore difficult to construct rational maintenance dose schedules and optimal dosage must be based on repeated determinations of the plasma concentration

KEY WORDS Neonate phenobarbital seizures pharmacokinetics

The incidence of convulsions during the first weeks of life has been reported to be 0 2–0 8% (13) The etiology is manifold Some pathological conditions causing convulsions can be treated specifically the importance of diagnostic procedures to reveal hypoglycemia electrolyte disturbances, central nervous system infections or metabolic disorders has been stressed by many authorities (17 10 6 7) In the majority of cases treatment will be symptomatic, though In many pediatric textbooks phenobarbital is suggested as treatment for convulsions in newborns, however the dosage regimens vary considerably The information regarding phenobarbital pharmacokinetics in newborn infants has been very limited Whereas disappearance of prenatally administered phenobarbital has been reported on (14 9) no data on blood concentrations of phenobarbital during treatment for neonatal convulsions have been published earlier

The present investigation was undertaken to gain basic information about phenobarbital absorption and disappearance from plasma in the newborn infant Such information seems to be a prerequisite for a rational treatment Primarily the intention was to determine plasma concentrations as a function of time after the administration of varying doses of the drug the collected data have however also led to some discussion regarding the anticonvulsive effect

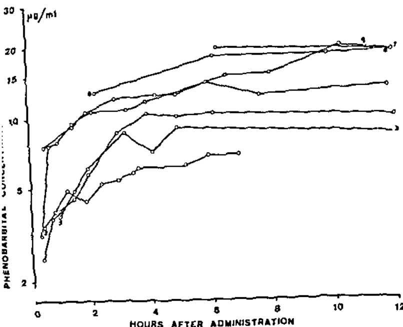


Fig 1 Phenobarbital plasma concentration in 7 infants during the first 12 hours after one oral dose Figures refer to patient numbers Broken lines indicate that the last part of the curve is based on continued determinations of plasma concentration represented in Figs 4 to 8 Individual doses are given in Table 2

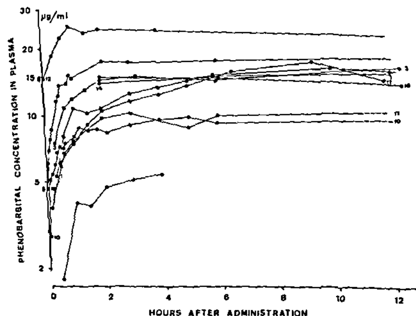


Fig 2 Phenobarbital plasma concentration in 10 infants during the first 12 hours after one intramuscular injection Arrows denote 2 infants whose mothers had been given phenobarbital before delivery as part of treatment for pregnancy toxemia Figures refer to patient numbers Broken lines indicate that the last part of the curve is based on continued determinations of plasma concentration represented in Figs 4 to 8 Individual doses are given in Table 2

Table 2 *First phenobarbital administration*

Pat no	Age h(ours)/ d(ays)	Dose (mg)	Ad min	Dose (mg/ kg)	Peak conc µg/ ml	hours after admin
1	2 d	7 5	p o	6 2/day	Not determined	
2	2 d	30	p o	7 3/day	6 9	7
3	2 d	50	p o	14 3	9 0	5*
4	17 h	45	p o	17 9	10 3	4
5	22 h	30	p o	18 6	13 9	6
6	2 d	75	p o	19 4	19 2	10
7	21 h	60	p o	19 8	19 5	6
8	3 d	60	p o	20 6	19 0	14*
9	7 d	16	i m	5 0	5 4	4
10	2 d	20	i m	7 3	9 8	5
11	2 d	26	i m	7 5	10 3	3
12	2 d	30	i m	9 2	–ª	3
13	2 d	45	i m	11 4	15 7	12
14	7 h	44	i m	12 0	14 9	6*
15	1 d	50	i m	12 0	15 6	10
16	7 h	45	i m	12 4	14 9	2*
17	3 d	28	i m	13 0	16 9	9
18	10 d	30	i m	13 2	–ª	3*

ª Incomplete sampling during the first 6 hours
* *Phenobarbital given to the mother before delivery*

samples were collected 20 40 60 90 and 120 minutes as well as 3 4 5 6 and about 24 hours after the dose thereafter once or twice daily for a period of varying length The duration of the individual sampling period can be seen in Figs 4 to 8 except for case 2 followed for 2 weeks and case 9 studied for only 24 hours Deviations from the routine sampling during the first 6 hours are noted in Table 2

In seven infants diagnostic lumbar puncture was performed and cerebrospinal fluid (CSF) could be obtained for the determination of phenobarbital concentration In two cases CSF was collected twice

Phenobarbital assay

The concentration of phenobarbital in plasma or CSF was determined in duplicate samples of 100 µl volume by means of gas liquid chromatography as previously described (11)

Protein assay

The protein concentration in cerebrospinal fluid was determined according to the method described by Lowry et al (12)

Pharmacokinetic analysis

In cases where the final slope of the plasma curves showed mono exponential decline of phenobarbital concentration the disappearance of the drug from the plasma has been mathematically characterized Application of the least squares method gives the disposition rate constant (16) The half life is then calculated by the equation

$$\text{Half life (hours)} = \frac{0\ 693}{\text{disposition rate constant (hours}^{-1})}$$

In cases where this calculation has been made after the first dose given extrapolation backwards in time until the moment of administration (time 0) gives the apparent concentration at time 0 The apparent volume of distribution is a mathematical expression relating the plasma concentration to the total amount of drug in the body It is influenced both by properties of the drug such as protein binding and lipid solubility and by factors such as body water composition and plasma protein concentration in the patient It is given by the equation

$$\text{Volume of distribution (l/kg)} = \frac{\text{dose (mg/kg)}}{\text{apparent concentration at time 0 } (\mu g/ml)}$$

RESULTS

Absorption of the first dose

Individual plasma curves in patients 2 to 18 during the first 12 hours after the administration of the first dose are given in Figs 1 and 2 At least 90% of the peak concentration was reached within 4 hours in 8 of 10 infants given intramuscular injections whereas the absorption after oral administration was considerably slower The relation between dose and peak concentration in plasma is graphically represented in Fig 3 After intramuscular injection the peak concentration (in µg/ml) roughly equalled 1 3× the dose (in mg/kg) After oral administration the peak concentration was lower *in relation* to the dose Individual doses and peak concentrations as well as the time lapse between administration and peak concentration are given in Table 2

Plasma concentrations after daily doses

Patient no 1 presented in Fig 5 illustrates the routine administration practised when this study began Plasma concentration increased during at least 5 days of treatment The same applied to patient no 2

Distribution and elimination

The individual plasma concentration curves are given in Figs 4 to 8 and results of the pharmacokinetic analyses are given in Table 3 Fig 4 shows which parts of the plasma curves have been used for the calculations In the cases presented in Figs 6 to 8 all determinations of the plasma concentration from 24 hours

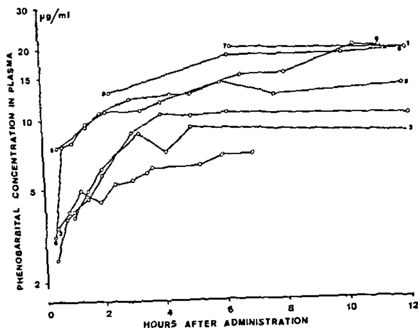


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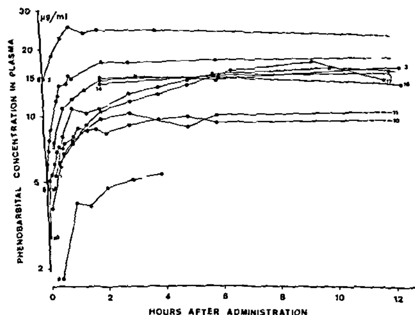


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9	7 d	16	i m	5 0	5 4	4
10	2 d	20	i m	7 3	9 8	5
11	2 d	26	i m	7 5	10 3	3
12	2 d	30	i m	9 2	–[b]	1
13	2 d	45	i m	11 4	15 7	12
14	7 h	44	i m	12 0	14 9	6
15	1 d	50	i m	12 0	15 6	10
16	7 h	45	i m	12 4	14 9	2[a]
17	3 d	28	i m	13 0	16 9	9
18	10 d	30	i m	13 2	–[b]	3[a]

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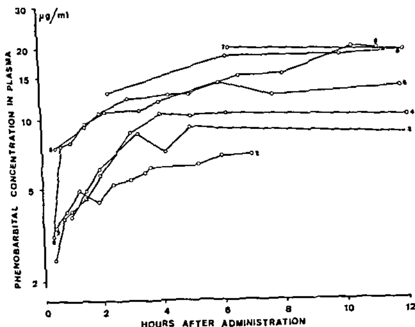


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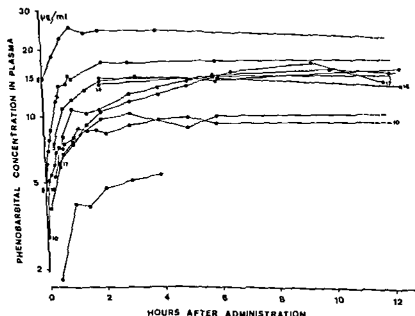


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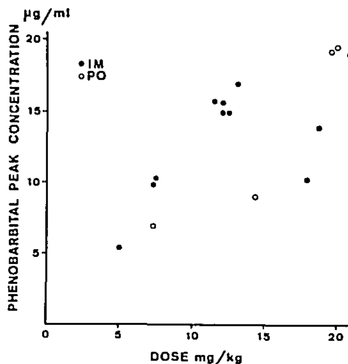


Fig 3 Relation between dose and peak concentration Dots in open symbols denote 2 infants who vomited shortly after oral administration

after the dose and onwards have been included in the calculations

The phenobarbital half life varied between 41 and 182 hours if values based on less than four plasma concentration determinations were excluded, the range was 59–182 hours

Some infants showed a considerable day to-day variation in the rate of disappearance from plasma as seen in Figs 4 to 8 In spite of this, pharmacokinetic analysis has been performed in all cases In 2 patients (nos 13 and 16) blood urea was increased for several days indicating renal insufficiency In all cases pH in peripheral blood was normal when routinely checked during the first days of the phenobarbital treatment period

In 2 cases (nos 7 and 10) a two-phasic disappearance curve was found the half lives calculated from the first parts of these curves are not included in the range mentioned above In patient no 3 only part of the curve was used for pharmacokinetic analysis The infant had a generalized herpes simplex virus infection and for unknown reasons the concentration of phenobarbital in plasma did not change for a couple of days The apparent volume of distribution after intramuscular injection varied between 0 59 and 0 84 l/kg (Mean 0 71 S D 0 10) After oral administration it was 0 89–1 54 l/kg

Phenobarbital in cerebrospinal fluid

In Table 4 the results of the cerebrospinal fluid analyses are arranged according to the space of

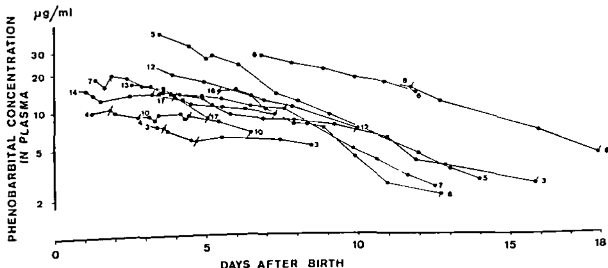


Fig 4 Plasma curves in infants not presented individually in Figs 5 to 8 All phenobarbital determinations from 12 hours after the last acute treatment dose are presented Arrows denote the limits of the part of each curve used for the calculation of the disposition rate constant and half life

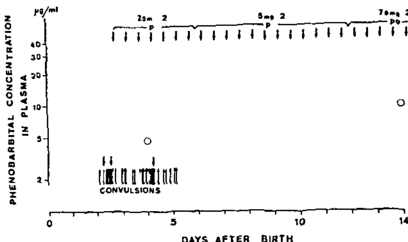


Fig 5 Patient no 1 This small for-date boy was given an albumin-saline infusion in exchange for 40 ml blood at 8 hours after birth when hematocrit was found to be 87% He had his first convulsion 50 hours after birth (opisthotonus and general clonic convulsions) Hydrocortisone by injection was given days 3 to 11 for treatment of hypoglycemia Phenobarbital treatment was started as convulsions continued in spite of glucose infusion The dose was decreased for a couple of days because of a suspected sedative effect Arrows on top of the diagram represent phenobarbital administration Arrows marked D diazepam injections Arrows on bottom convulsions Symbols enclosed by circle denote phenobarbital concentration in CSF

time between the first administration of the drug and the lumbar puncture In patients 2 and 3 where lumbar puncture was performed only 40 minutes after oral administration no phenobarbital was found in the CSF (The lower limit for phenobarbital determination is approximately 0 5 µg/ml) From 17 hours after intramuscular injection up to after 12 days of

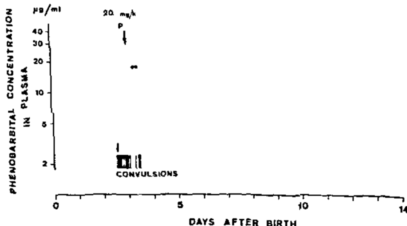


Fig 6 Patient no 8 Although the delivery was reported to proceed quickly but normally Apgar score was only 2 after 3 minutes and 7 after 5 minutes Ten minutes after birth all vital functions were considered satisfactory Thirty hours after birth the boy had a spell of apnoea and cyanosis and was brought to the neonatal ward Seizures (chewing twitchings around the eyes clonic movements of the right hand) appeared at the age of 2½ days and disappeared 9 hours after administration of phenobarbital He had no other neurological symptoms and was not noticeably sedated EEG on the ninth day of life showed epileptogenic activity and longterm phenobarbital treatment was given from the fourteenth day of life Symbols as in Fig 5

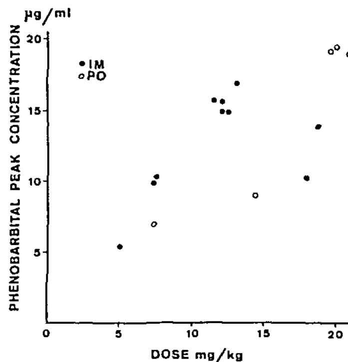


Fig 3 Relation between dose and peak concentration Dots in open symbols denote 2 infants who vomited shortly after oral administration

after the dose and onwards have been included in the calculations

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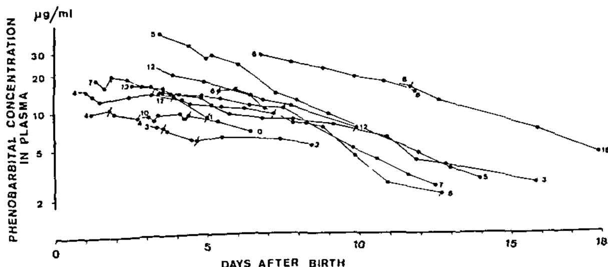


Fig 4 Plasma curves in infants not presented individually in Figs 5 to 8 All phenobarbital determinations from 12 hours after the last acute treatment dose are presented Arrows denote the limits of the part of each curve used for the calculation of the disposition rate constant and half life

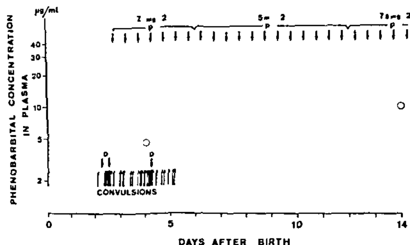


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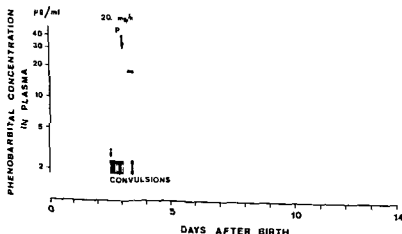


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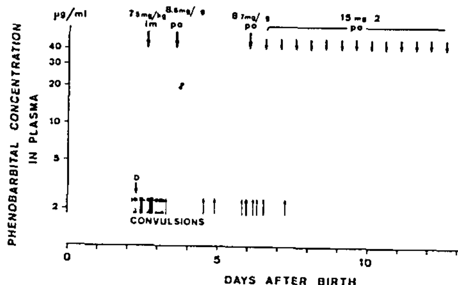


Fig 7 Patient no 11 After an uneventful pregnancy and normal delivery this boy had generalized tonic–clonic convulsions from his third day of life Repeated EEGs showed epileptogenic activity Long term phenobarbital treatment was instituted on the seventh day of life After a recurrence of seizures at 3 months of age he is still on anticonvulsive treatment at 2 years of age Symbols as in Fig 5

daily treatment the CSF/plasma ratio varied between 0 48 and 0 83

Relation between plasma concentration and effect

In 8 cases (nos 1 2 5 7 8 11 15 16) an apparent relation between the concentration of phenobarbital in plasma and the disappearance of convulsions was found Four of them are presented in Figs 5 to 8 The approximate plasma concentration when convulsions ceased ranged between 12 and 20 µg/ml with the exception of patient 11 (Fig 7) where after a relapse the convulsions did not disappear until the concentration had reached nearly 30 µg/ml

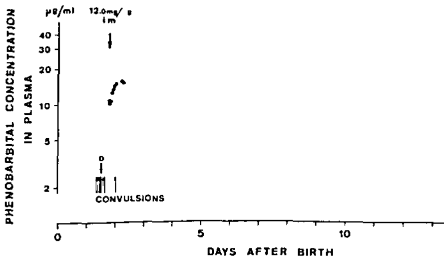


Fig 8 Patient no 15 Vacuum extractor was applied because of slow progress during the last part of delivery foetal heart rate was never depressed The boy had no spontaneous breathing but a regular heart activity immediately after birth After resuscitation breathing was regular at the age of 15 min His tonus was then found to be increased There were 1500 red cells/mm³ in the CSF On the second day of life he had convulsions (opisthotonus clonic movements in left arm and leg) the last one appearing 7 hours after the administration of phenobarbital At 2 weeks of age he was considered neurologically normal No explanation for the day to-day variations in the rate of phenobarbital disappearance from the plasma has been found Symbols as in Fig 5

Table 3 *Phenobarbital distribution and disposition*

Pat no	Presented in Fig no	Disposition rate constant (hours⁻¹)	Half life (hours)	Apparent conc at time 0 (μg/ml)	Apparent volume of distribution (l/kg)	Remarks
3	4	0 0091	76	9 3	1 54	Disposition rate constant calculated from part of the plasma curve only
4	4	0 0061	114	12 1	1 48	Disposition rate constant calculated from only three determinations of plasma concentration
5	4	0 0109	64			Three doses given Disposition rate constant determined after the third dose
6	4	(*a*) 0 0079 (*b*) 0 0053	88 131	21 9	0 89	Four doses given Disposition rate constant determined after the first (*a*) and the fourth (*b*) dose
7	4	0 0046/ 0 0108	151/64	20 7	0 96	Two-phasic disappearance curve the apparent concentration at time 0 calculated from the first part
8	6	0 0084	83	21 0	0 98	
10	4	0 0005/ 0 0060	1 386/116	8 9	0 82	Two-phasic disappearance curve the apparent concentration at time 0 calculated from the first part
11	7	0 0048	144			Disposition rate constant calculated after the second dose from only three determinations of plasma concentration
12	4	0 0069	100			Phenobarbital given to the mother before delivery
13	4	0 0058	119	17 8	0 64	
14	4	0 0040	173	18 1	0 66	
15	8	0 0113	61	20 2	0 59	
16	4	(*a*) 0 0038 (*b*) 0 0117	182 59	14 7	0 84	Two doses given Disposition rate constant determined after both the first (*a*) and the second (*b*) dose
17	4	0 0167	41	19 2	0 68	Disposition rate constant calculated from only two determinations of plasma concentration
18	4	0 0080	87			Phenobarbital given to the mother before delivery

Cannot be established disposition rate constant not determined after the first dose

In 4 infants (patients 4 6 12 and 17) phenobarbital did not seem to influence the frequency of seizures Maximum plasma phenobarbital concentration in these cases were 10 3 32 3 25 7 and 16 9 μg/ml respectively

In 6 infants the clinical course did not provide any basis for an attempt to evaluate the anticonvulsive effect of phenobarbital patients 9 and 18 had convulsions only before the administration of the drug in patients 3 10 13 and 14 the suspected seizures appeared as clonic movements or apnoeic spells and were never confirmed to be the expression of abnormal electric brain activity

DISCUSSION

Convulsions in newborns are often less dramatic and of shorter duration than those in older children Nevertheless most authors advocate anticonvulsive treatment In these instances

Table 4 *Phenobarbital in cerebrospinal fluid*

Pat no	Sex	Age (days)	Route of administration	Interval between admin and lumbar puncture	Phenobarbital concentration in CSF (μg/ml)	in plasma (μg/ml)	ratio CSF/plasma	Protein in CSF (mg/100 ml)
2	M	2	p o	40 min	N M	4 0	–	N A
3	F	2	p o	40 min	N M	3 0	–	N A
16	M	1	i m	17 hours	9 8	12 4	0 79	68
3	F	3	p o	22 hours	6 2	7 5	0 83	368
6	M	3	p o	27 hours	10 8	17 8	0 61	122
14	M	2	i m	50 hours	7 2	13 5	0 53	140
1	M	4	p o	3 doses given 30 18 and 6 h before sampling	4 7	7 4	0 64	84
1	M	14	p o	Daily doses for 12 days	9 9	20 5	0 48	55
4	F	21	p o	Daily doses for 12 days	12 6	21 7	0 58	31

Estimated value interval between lumbar puncture and plasma sampling 10 minutes
N M Not measurable
N A Not analysed

phenobarbital should obviously not be given in repeated small doses resulting in a slow approach to steady state but in a relatively large first dose aiming towards a therapeutic drug concentration in the central nervous system Thus one of the main purposes of the present investigation was to find out how quickly and how reliably phenobarbital is absorbed in the neonatal period In older infants absorption takes place to the same degree and almost as quickly after oral administration as after intramuscular injection (8) In the often seriously ill newborns in the present report, the drug was absorbed more reliably and considerably quicker after injection than after ingestion

In adults the range of anticonvulsive therapeutic concentration of phenobarbital is reported to be 10–25 μg/ml (1) The only report on therapeutic concentration in children is the one by Faerø et al (4) They found that serum concentrations over 15 μg/ml protected against relapses of febrile convulsions The youngest infant in their investigation was however 4 months old In animal experiments the degree of penetration of phenobarbital into the central nervous system has been found to vary with age (2 5) and from *in vitro* studies on the binding to human plasma protein it is known that plasma from newborns binds phenobarbital to a lesser degree than plasma from adults (3) It is therefore possible that a certain plasma concentration corresponds to different cerebral tissue concentrations in different age groups

In the present study a close relation in time was found in some of the patients between the disappearance of the convulsions and the achievement of phenobarbital plasma concentration in the range 12–30 μg/ml One has to be very careful when drawing conclusions regarding the anticonvulsive effect in the newborn period though as seizures in neonates often disappear spontaneously within a couple of days Moreover in this investigation there is no control group of infants given other or no anticonvulsive treatment However the results seem to suggest that phenobarbital has an anticonvulsive effect in newborn infants when such plasma concentrations are reached that correspond to the range reported to be therapeutic in older infants and adults (1 4)

Side effects were difficult to evaluate in the present investigation Very probably sedation occurred in several of the infants given high initial doses but in the individual case it was often impossible to judge whether the disease causing the convulsions or the treatment brought about the depression of spontaneous

activity Breathing was never seriously affected Further information concerning the relation between the plasma concentrations and the side effects could possibly be collected in newborns with less dramatic clinical conditions as for example infants given phenobarbital as prophylaxis for hyperbilirubinemia.

The final slope of the plasma curve on which the pharmacokinetic analysis was based mainly represents metabolism and excretion of the drug The half life of phenobarbital varied between 59 and 182 hours in the present study No correlation could be found between the half life and the length of gestation birth weight post partum age or the administration of other drugs to mother or infant Probably the most important factor influencing both the metabolism and the elimination of the drug in these infants were their primary diseases

Some interesting phenomena were met within the interpretation of the individual plasma curves In some cases the rate of disappearance varied considerably from day to day In only 2 of these infants (patients 13 and 16) can a possible explanation for this variation be suggested as the renal function was reduced during part of the observation period Variations in plasma pH are known to influence the distribution between the plasma and the tissues (18) but was not found in any of the infants concerned The conditions causing convulsions in the newborn period are however often so serious that disturbances in the circulation metabolism and elimination are to be expected

In two instances (patients 7 and 10) biphasic phenobarbital disappearance curves from the plasma were found similar plasma curves have been reported earlier in 3 of 18 infants in whom we followed the disappearance from plasma of prenatally administered phenobarbital (9)

Even in infants who later had very small day to-day variations in the rate of disappearance of phenobarbital from plasma there were considerable fluctuations during the first 24 hours (Figs 4 to 8) What part of the plasma curve that could be considered to show a mono-exponential concentration decline (and that could thus serve as a basis for the pharmacokinetic analysis) has therefore been decided individually in each case

The volume of distribution of phenobarbital in older infants has been shown to vary between 0 47 and 0 76 l/kg after intramuscular injection (8) The few data collected on this point in the present investigation suggest that newborn infants have a somewhat larger volume of distribution In older infants the ratio between the peak concentration (in µg/ml) and dose (in mg/kg) was 1 6 (8) in the present study it was 1 3 after intramuscular injection These findings are in agreement with a higher volume of distribution in the newborns The high values found after oral administration are probably partly explained by an incomplete absorption Again however disease might have influenced also the distribution the largest volume of distribution was found in patient no 3, who had a high ratio between the concentration of phenobarbital in CSF and plasma probably because her disease caused an increased passage through the blood–liquor barrier

The considerable inter individual differences in the rate of disappearance of phenobarbital from plasma in newborns with convulsions make it difficult to construct safe dosage regimens for maintenance therapy This difficulty is even more pronounced in cases where the rate of disappearance is not constant which seems to be not unusual If we want to keep the plasma concentration within fairly close limits we shall probably have to rely upon regular determinations of the plasma concentration to check and steer our treatment

ACKNOWLEDGEMENTS

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REFERENCES

1 Buchthal F & Lennox Buchthal M A Phenobarbital Relation of serum concentration to control of sei

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GASTRIC EMPTYING IN NEWBORNS AND YOUNG INFANTS

Measurement of the Rate of Emptying Using Indium 113m Microcolloid

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ABSTRACT Signer E and Fridrich R (University Children s Hospital and the Department of Nuclear Medicine Kantonsspital Basel Switzerland) Gastric emptying in newborns and houng infants Acta Paediatr Scand 64 525 1975.—The rate of gastric emptying was measured in newborns and young infants by a new radio-isotopic method 28 control babies and 6 infants with projectile vomiting were given a 50 ml standard milk feeding containing 15 μCi of Indium 113m microcolloid The radioactivity in the stomach was counted at regular intervals with a gamma-camera The gastric emptying followed an exponential pattern with a half-life of 87±29 minutes in 24 out of 28 control babies In 6 patients with projectile vomiting gastric emptying was impaired severely Three of them with hypertrophic pyloric stenosis showed complete stasis of gastric contents Gastric emptying returned to normal 8–16 days after pyloromyotomy It is suggested that the radioisotopic technique is helpful in evaluating the severity of pyloric stenosis and that it is of value in studies of the action of pharmacological substances on gastric emptying

KEY WORDS Gastric emptying time Indium 113m microcolloid newborns infants pyloric stenosis

Knowledge of gastric emptying in childhood is very limited and little progress has been made in this subject over the past twenty years Until recently most studies have made use of either serial test meal technique (2 13 14) or the X ray method following a barium meal (1 9 11 15) Unfortunately both methods are unsatisfactory especially in infants and children and are not often used in the paediatric age group

In 1966 Griffith et al (4 5) introduced a new radioisotopic technique to determine the rate of gastric emptying in adults A standard meal was labelled with radioactive chromium (^{51}Cr) and the rate at which it left the stomach was measured by external counting This method made it possible to study the quantitative aspects of gastric emptying under physiological conditions without any discomfort to the patient The usefulness of the radioisotope technique was confirmed by Harvey et al (8) and Fridrich et al (3) who modified the method by using radioactive Indium (^{113m}In) and a gamma camera In this communication we describe the application of the latter technique to the infant age group

SUBJECTS

The following subjects were studied

(a) Control group

This group consisted of 28 newborns and young infants (including 3 premature babies) aged 1–10 weeks mean age 25 days who were free from primary or secondary gastrointestinal symptoms No medicaments were given from 3 days before the investigation

zures *In* D M Woodbury J K Penry & R P Schmidt (eds) *Antiepileptic drugs* Raven press New York 1972 p 335
2 Domek N S Barlow C F & Roth L J An ontogenetic study of phenobarbital – C^{14} in cat brain *J Pharmacol Exp Ther 130* 285 1960
3 Ehrnebo M Agurell S Jalling B & Boréus L O Age differences in drug binding by plasma proteins Studies on human foetuses neonates and adults *Eur J Clin Pharmacol 3* 189 1971
4 Faerø O Kastrup K W Lykkegaard Nielsen E Melchior J C & Thorn I Successful prophylaxis of febrile convulsions with phenobarbital *Epilepsia 13* 279 1972
5 Ferngren H Brain and blood levels of phenobarbital 2 ^{14}C during postnatal development in the mouse *Acta Pharm Suec 6* 331 1969
6 Freeman J M Neonatal seizures—diagnosis and management *J Pediatr 77* 701 1970
7 Gamstorp I *Paediatric Neurology* Appleton Century Crofts New York 1970 p 91
8 Jalling B Plasma and cerebrospinal fluid concentrations of phenobarbital in infants given single doses *Dev Med Child Neurol* in press
9 Jalling B Boréus L O Källberg N & Agurell S Disappearance from the newborn of circulating prenatally administered phenobarbital *Eur J Clin Pharmacol 6* 234 1973
10 Keuth U Neugeborenenkrampfe *Monatsschr Kinderheilk 117* 428 1969
11 Källberg N Agurell S Jalling B & Boréus L O Rapid gas chromatographic determination of phenobarbital in small plasma samples *Eur J Clin Pharmacol 3* 185 1971
12 Lowry O H Rosebrough N J Farr A L & Randall R J Protein measurement with the folin phenol reagent *J Biol Chem 193* 265 1951
13 McInerny T K & Schubert W K Prognosis of neonatal seizures *Am J Dis Child 117* 261 1969
14 Melchior J C Svensmark O & Trolle D Placental transfer of phenobarbitone in epileptic women and elimination in newborns *Lancet II* 860 1967
15 Norell E & Gamstorp I Neonatal seizures effect of lidocain *Acta Paediatr Scand* Suppl *260* 97 1970
16 Riegelman S Loo J C K & Rowland M Shortcomings in pharmacokinetic analysis by conceiving the body to exhibit properties of a single compartment *J Pharm Sci 57* 117 1968
17 Schwartz J F Neonatal convulsions Pathogenesis diagnostic evaluation treatment and prognosis *Clin Pediatr 4* 595 1965
18 Waddell W J & Butler T C The distribution and excretion of phenobarbital *J Clin Invest 36* 1217 1957

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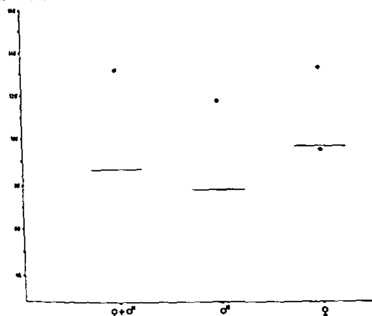


Fig 2 Distribution of the half life of a standard milk meal in newborns and young infants without gastroenterological disease Horizontal lines = mean half life ● full term babies O premature babies

ings The sixth patient (B D) displayed a normal X ray pattern with the exception of a slow emptying of the barium meal Subsequent bacteriological investigation indicated gastro enteritis due to *E coli* 0 127 In all 6 patients the gastric emptying time was measured before and after treatment

Four infants with severe pyloric stenosis showed no gastric emptying at all (observation period 90–110 min) indicating almost complete obstruction of the gastric outlet Three of them (M O G S F H) underwent subsequent surgical treatment with pyloromyotomy The gastric emptying reached normal values 8–16 days after the operation (Fig 5)

Patient G M was sent to the hospital with a

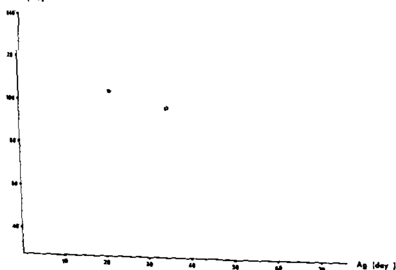


Fig 3 Distribution of the half life in relation to age No correlation between half life and age ($r = 0.043$) ● full term babies O premature babies

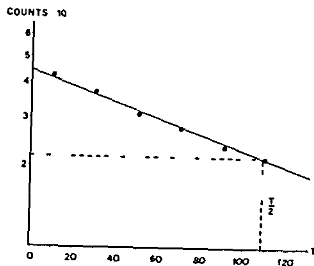


Fig 1 Gastric emptying (monophasic type) Disappearance of 113 m Indium from the stomach of a normal infant (Subject B N aged 32 days) The emptying pattern follows a single exponential function Half life ($T/2$) = 109 min

(b) Patients with projectile vomiting

Six infants aged 4–10 weeks admitted to the hospital with a presumptive diagnosis of hypertrophic pyloric stenosis were studied twice before and after treatment

Permission to study these subjects was obtained from the parents after a full explanation of the procedure

METHODS

In each subject the rate of gastric emptying was measured as follows

After a fasting time of 6 hours a standard bottle feeding was given consisting of 50 ml of an adapted cow s milk formula (Humana 1[1])

15 μCi of Indium 113m gelatine were incorporated into the milk as a microcolloid After the bottle feeding the infants were allowed to regurgitate swallowed air and then placed in a supine position under the detector of the gamma camera (Picker Dynacamera) The time interval between the beginning of the meal and the first counting was usually 10–20 min The amount of radioactivity in the stomach was measured at regular intervals (10–20 min) Corresponding to the source of the gamma rays a scintigraphic picture of the stomach was printed on the oscilloscope screen of the gamma camera By this means a region of interest containing the whole stomach could be outlined The impulses from this region were then counted during a period of 100 sec with the aid of a multi-channel analyser Since most infants were sleeping quietly after the feed keeping the same region of interest during all measurements was no problem It is generally accepted that the rate at which food and fluids leave the stomach can be expressed by a single exponential function By plotting

Table 1 *Gastric emptying in normal infants* (Monophasic Type)

Subjects	Number	Half Life (min) Mean	S D	Range
Total	24	87	29	45–141
Boys	12	77	23	5?–117
Girls	12	96	32	45–145

the measured counts corrected with physical decay on semilogarithmic paper against the time a straight line was usually obtained from which the half life was directly read (Fig 1) The latter index has been used in this study to describe the rate of gastric emptying in a quantitative manner

RESULTS

(a) Control group

Two different types of gastric emptying could be observed

1 *Monophasic type* (Fig 1) In 24 of the 28 control babies the stomach emptied in a single exponential manner during the 90–100 min period following the first measurement at 10–15 minutes after the meal Values of $T/2$ ranged from 45–141 minutes and were evenly distributed around the mean (mean 87 minutes S D ±29 min) (Table 1 Fig 2) No significant difference was found between the mean values of the male and female group ($t=1\ 68$ $p>0\ 1$) Little correlation was found between the age of the infants and the corresponding $T/2$ values ($r=0\ 043$ Fig 3)

2 *Diphasic type* (Fig 4) In 4 out of the 28 control subjects the rate of gastric emptying did not follow a single exponential curve but occurred in two distinct phases During phase 1 stasis of the gastric contents was indicated by a very long half life In phase 2 the rate stomach emptying was similar to that in t group of monophasic type (Table 2)

(b) *Patients with projectile vomiting* (Table :

In 6 patients aged 4–10 weeks the clinic diagnosis of hypertrophic pyloric stenosis wa made In 5 of them the pyloric stenosis wa confi

[1] Humana 1 contains 1 7 g protein 3 7 g fat and 7 2 g lactose per 100 ml reconstituted feed Producer Schweiz Milch-Gesellschaft CH 6280 Hochdorf (Switzerland)

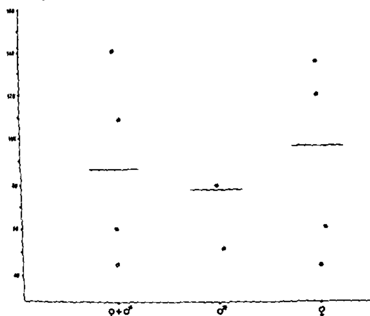


Fig 2 Distribution of the half life of a standard milk meal in newborns and young infants without gastroenterological disease Horizontal lines = mean half life ● full term babies O premature babies

ings The sixth patient (B D) displayed a normal X ray pattern with the exception of a slow emptying of the barium meal Subsequent bacteriological investigation indicated gastroenteritis due to *E coli* 0 127 In all 6 patients the gastric emptying time was measured before and after treatment

Four infants with severe pyloric stenosis showed no gastric emptying at all (observation period 90–110 min) indicating almost complete obstruction of the gastric outlet Three of them (M O G S F H) underwent subsequent surgical treatment with pyloromyotomy The gastric emptying reached normal values 8–16 days after the operation (Fig 5)

Patient G M was sent to the hospital with a

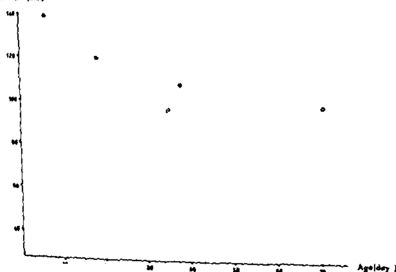


Fig 3 Distribution of the half life in relation to age No correlation between half life and age (=0 043) ● full term babies O premature babies

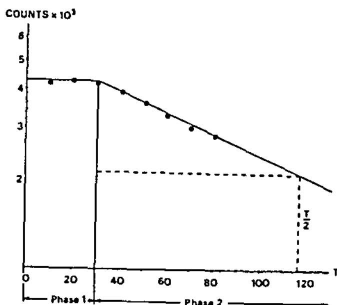


Fig 4 Gastric emptying (diphasic type) Phase 1 Stasis of gastric contents during 30 min Half life ($T/2$)=∞ Phase 2 Exponential emptying pattern Half life ($T/2$)=86 minutes (Subject M P aged 10 days)

Table 2 *Gastric emptying in normal infants* (Diphasic Type)

Subject	Phase 1 Half-life (min)	Phase 1 Duration (min)	Phase 2 Half life (min)
H M	∞	30	105
M P	∞	30	87
G E	∞	40	45
M G	>200	35	55

relapse of pyloric stenosis 8 weeks after a previous pyloromyotomy To avoid a second operation medical management was tried (12 meals per day methylscopolamine and phenobarbitone) Vomiting subsided but after 5 weeks of treatment the gastric emptying was still very slow with a half life of 160 min

Patient G J exhibited on clinical and roentgenological grounds a slight form of pyloric stenosis without complete obstruction In agreement with this a diphasic emptying pattern was found Before treatment the stomach emptied during the first 40 min at a normal rate but subsequently the emptying process stopped completely and the radioactivity in the stomach remained constant during a further 60 min Following medical management the gastric emptying rate was still very slow in the first phase ($T/2$=240 min) but normal in the second phase ($T/2$=70 min)

In the patient with *E coli* gastroenteritis (B D) the symptoms disappeared gradually In 15 days the half life which was 328 min at the beginning improved to a normal rate of 60 min

DISCUSSION

Information on the physiology and pathophysiology of gastric emptying in paediatrics is still very rare in spite of many clinical situations in which a better knowledge of the pattern and rate of gastric emptying would be of great value The present lack of information is mainly due to the unsatisfactory methods of measuring the rate of gastric emptying

Until recently the most reliable method for studying the rate of gastric emptying was the *serial test meal technique* This technique, introduced by Salamanca (2) Hunt & Spurrell

Table 3 *Gastric emptying in infants with projectile vomiting*

Subject	Diagnosis	Therapy	Half life (min) Before Therapy	Half life (min) After Therapy	Interval (days)
M O	Pyloric stenosis	Surgery	∞	110	14
G S	Pyloric stenosis	Surgery	∞	48	16
F H	Pyloric stenosis	Surgery	∞	110	8
G M	Pyloric stenosis (relapse)	Medication	∞	160	33
G U	Pyloric stenosis (slight)	Medication	57 (40)/∞	240 (60)/70	37
B D	Gastroenteritis (E coli)	Medication	328		

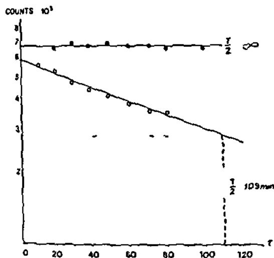


Fig 5 Gastric emptying in pyloric stenosis ●—● before and O—O after pyloromyotomy Before pyloromyotomy stasis of gastric contents during 110 min Half life (T/2)=∞ Following therapy normal emptying pattern Half life (T/2)=109 min (Patient F H age 28 resp 36 days)

(13 14) involves giving a series of standard test meals on several days which are recovered after different time intervals by repeated nasogastric intubation This method has several disadvantages only fluid meals can be given the repeated intubations are disagreeable and may alter the physiology of gastric emptying In paediatric patients apprehension and excitement during intubation may considerably influence the results Furthermore since a prerequisite for this technique is a constant emptying rate from day to day studies of acute conditions are almost impossible

To avoid serial intubations gastric emptying was measured by *X ray studies after a barium meal* (1 11) and what we know about gastric emptying in infants and children is almost completely derived from early roentgenologic studies However by using this method it is extremely difficult to obtain reliable quantitative data. The estimation of the final emptying time defined as the time taken for barium to leave the stomach completely is particularly meaningless In normal infants barium residues can be found commonly 8–12 hours after feeding Henderson (11) mentions the frequency with which he found barium still present 24 hours after feeding Fridrich et al (3) showed that in adults there is no correlation between the half life measured by the radioisotope method and the roentgenological emptying time This may be due to the tendency of barium sulfate to separate from the food and to leave the stomach at a different rate as well as to the adherence to the gastric mucosa (6) Attempts to obtain more reliable data by estimation (9) or planimetric measurement of the radioopaque gastric areas on radiographs taken at several intervals are not only inaccurate but result in considerable exposure of the patients to radiation

With the *radioisotope technique* used in this study detailed information on the gastric emptying process can be obtained without disturbance to the patient This method therefore is particularly useful in infants and children Solid meals can be given as well as fluid ones As shown in adults (3 6) the results are highly reproducible With the use of Indium 113m microcolloid the radiation dose is very low With an activity of 15 μCi In 113m microcolloid the stomach dose is 12 mrads the gonads dose is lower than 0 1 mrad (10)

Our results show that gastric emptying in most of the newborns and young infants follows a single exponential pattern during a period of 10–100 min after a milk feeding The mean T/2 of 87 min is considerably longer than found in normal adults where the mean T/2 was 65 min (6) The general tendency in early life for feed to leave the stomach slowly is well known and has been attributed to such factors as poorly developed gastric musculature with shallow and irregular contractions gaseous distention of the stomach and recumbent supine position (16) Although in our study great care was taken to promote the regurgitation of swallowed air a variable gas content in the stomach may explain the wide range in normals It is probable that swallowed air also contributed to the stasis of gastric contents found in four babies with a diphasic emptying pattern The factors of air content in the alimentary tract and the position

of the infant must therefore be taken into account in the evaluation of the gastric emptying pattern (12)

It was somewhat surprising that no correlation could be demonstrated between the half life and the age of the infants during the first 6 weeks of life This may be due to the relative small number of tests and the wide variation of half life at this age It is possible that a correlation may be found by comparing a larger range of age groups

In severe cases of *hypertrophic pyloric stenosis* the radioactivity in the gastric area remained constant during a period of 90–110 min after the meal indicating stasis of gastric contents 8–16 days following pyloromyotomy the rate of gastric emptying had returned to normal On the other hand even after 5 weeks of conservative therapy the half life remained prolonged Radioisotope measurement of gastric emptying although not necessary for the diagnosis of pyloric stenosis can be helpful in evaluating the severity of the condition and would indicate which patients need surgery

In one of the patients with projectile vomiting delayed gastric emptying was associated with an *E coli* gastroenteritis Although this situation requires further investigation it is possible that the vomiting in acute gastroenteritis is due to severe disturbance of the gastric emptying process

There is no doubt that the radioisotope method will be of special value in studies of the action of pharmacological substances upon the rate of gastric emptying as has already been demonstrated in adults (7)

REFERENCES

1 Bouslog J S Cunningham T D Hanner J P Walton J B & Waltz H D Roentgenologic studies of infants gastrointestinal tract *J Pediatr 6* 234 1935

2 De Salamanca F E Estudio de fisiologia gastrica. *Arch Med Exp* (Madrid) *3* 17 1949

3 Fridrich R Stalder G A Locher J & Heinis P *Zur Erweiterung der Magendiagnostik mit nuklear medizinischen Verfahren Dtsch Med Wochenschr 95* 2261 1970

4 Griffith G H Owen G M Kirkman S & Shields R Measurement of rate of gastric emptying using chromium 51 *Lancet I* 1244 1966

5 Griffith G H Owen G M & Shields R The rate of gastric emptying in gastro-duodenal disease *Gut 7* 717 1966

6 Griffith G H Owen G M Campbell H & Shields R Gastric emptying in health and in gastroduodenal disease *Gastroenterology 54* 1 1968

7 Gyr N Berger W Fridrich R Denes A & Stalder G A Der Einfluss von Dimethylbiguanid auf die Magenentleerung und die orale Glukosetoleranz. *Schweiz Med Wochenschr 101* 1876 1971

8 Harvey R F Brown N J G Mackie D B Keeling D H & Davies W T Measurement of gastric emptying time with a gamma-camera *Lancet I* 16 *1970*

9 Hawkins G K Margolin S & Thompson J J Gastric emptying time comparative studies with placebos prantal and banthine *Gastroenterology 24* 193 1953

10 Heading R C Tothill P Laidlaw A J & Sherman D J G An evaluation of 113m Indium DTPA chelate in the measurement of gastric emptying by scintiscanning *Gut 12* 611 1971

11 Henderson S G The gastrointestinal tract in the healthy newborn infant *Am J Roentgenol 48* 30? 1942

12 Hood J H Effect of posture on amount and distribution of gas in the intestinal tract of infants and young children *Lancet II* 107 1964

13 Hunt J N & Spurrell W R The pattern of emptying of the human stomach *J Physiol* (London) *113* 157 *1951*

14 Hunt J N Gastric emptying and secretion in man *Physiol Rev 39* 491 1959

15 Sacchetti G Nicolis F B & Roncoroni L Evaluation in man of the effects of an anticholinergic agent on gastric emptying by a quantitative X ray method *J Pharmacol Exp Ther 145* 393 1964

16 Smith C A *The physiology of the newborn infant* Thomas Springfield Illinois 1946 2nd ed pp 166–173

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COMPLEMENT FIXING AND LYMPHOCYTOTOXIC ANTIBODIES IN SERUM OF PREGNANT WOMEN AT DELIVERY

III Clinical Observations

GUNNAR NYMAND

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ABSTRACT Nymand G (Regional Blood Transfusion Center Aalborg Denmark) Complement fixing and lymphocytotoxic antibodies in serum of pregnant women at delivery III Acta Paediatr Scand 64 531 1975.—The investigation comprised 2008 pregnancies. Sera from all the pregnant women were examined for complement fixing platelet antibodies (CFPAb) and lymphocytotoxic antibodies (LCTAb) 14 women had only CFPAb 44 had CFPAb and LCTAb 224 had only LCTAb In 3 of 481 cord blood serum samples LCTAb were demonstrated No cases of neutropenia or thrombocytopenia were observed No association was found between CFPAb or LCTAb and stillbirth birth defects degree of bilirubinaemia diseases of the newborn or placental weight It was suggested that the applied lymphocytotoxicity test detects both in vivo cytolytic antibodies and enhancing antibodies which may explain the results

KEY WORDS Newborns complement fixing antibodies lymphocytotoxic antibodies

Now it is generally accepted that fetoplacental antigens challenge the immune apparatus of the pregnant woman and provoke a humoral and a cellular immune response as newly reviewed by Ceppelini (8) The humoral response might be directed against cellular and non cellular antigens McKay & Thom (15) described maternal antibodies against fetal gammaglobulins Nearly all antibodies formed as a consequence of feto maternal incompatibility were found in the 7 S fraction and belong to the IgG classes (3 14 20 26 30) These antibodies are able to penetrate the placental barrier and reach the fetal circulation due to their Fc fraction properties (7) Many investigators (5 11 14) have documented how maternal leucoagglutinins may cause neonatal neutropenia Neonatal puerpura was shown to be a consequence of maternal complement fixing platelet antibodies (22) or antibodies against fetal megakaryocytes (4) Two cases of neonatal thrombocytopenia caused by maternal complement fixing platelet antibodies (CFPAb) and lymphocytotoxic antibodies (LCTAb) with HLA specificity were described by Colombani et al (9)

The aim of the present study was to elucidate the clinical relevance of CFPAb and LCTAb in maternal serum for the condition of the offspring

MATERIAL AND METHODS

The study comprised 2008 pregnancies and covered a period of one year Serum samples from the mothers were obtained at delivery and examined for the presence of LCTAb and CFPAb as described earlier (19) Cord blood samples were collected at birth All the women in the study were interviewed by the author The case reports of the children were reviewed 6 months after birth in order to obtain information about congenital defects and diseases not diagnosed at birth Statistical calculations were done by

Table 1 *Distribution of newborns according to stillbirth birth defects bilirubinaemia and po natal diseases with reference to antibody content in women s sera*
CFPAb=complement fixing platelet antibodies LCTAb=lymphocytotoxic antibodies

	Total		Women with CFPAb without LCTAb in serum		Women with CFPAb and LCTAb in serum		Women without CFPAb with LCT in serum	
	n	%	*n*	%	*n*	%	*n*	%
Stillbirth	10 of 2 008	0 5	0 of 14	0 0	2 of 44	4 5	0 of 2?4	0
Birth defects	117 of 2 008	5 8	1 of 14	7 1	4 of 44	9 1	14 of 224	6
Bilirubinaemia <100 μmol/l	1 295 of 1 994	64 9	6 of 14	42 9	25 of 42	59 5	142 of 223	63
Bilirubinaemia 100–300 μmol/l	688 of 1 994	34 5	7 of 14	50 0	16 of 42	38 1	81 of ?23	36
Bilirubinaemia >300 μmol/l	11 of 1 994	0 6	1 of 14	7 1	1 of 42	2 4	0 of ?23	0
Post natal diseases								
Asphyxia	19 of 1 951	1 0	0 of 14	0 0	0 of 40	0 0	2 of 218	0
Infections	24 of 1 951	1 2	1 of 14	7 1	1 of 40	2 5	0 of 218	0

the use of the chi square test with Yates correction for small numbers and Students *t* test as described by Hill (10) Serum bilirubin estimations were done by the method described by White et al (29) normal values are lower than 30 μmol per litre After cutting of the cord the newborns were weighed with an accuracy of ±20 g The placental weights indicated the weight of placenta with membranes and umbilical cord after the placenta had been passively drained of blood

RESULTS

Fourteen women had only CFPAb in their serum 44 women had CFPAb and LCTAb and 224 women had only LCTAb In 1 726 women s sera no CFPAb or LCTAb were detected Furthermore 481 cord blood serum samples were examined for LCTAb in 3 cases LCTAb were demonstrated 2 of these were demonstrably cytotoxic against the newborns own lymphocytes in the third case we were not able to test the serum against lymphocytes of the newborn All 3 children were in good health without any symptoms of leuco or thrombocytopenia The mother in one of the three cases had an operational monospecific anti HL A 9, she was pregnant for the first time and had never had blood transfusions Unfortunately, we could not check the HL A type of the child, as we were not allowed to take further blood samples The other 2 women had operationally polyspecific antibodies, as demonstrated in cord blood serum

In Table 1 the newborns are grouped according to the antibodies demonstrated in their mothers serum and distributed according to the occurrence of stillbirth birth defects degree of bilirubinaemia and diseases in the postnatal period When the various groups of newborns of women with antibodies were compared with newborns of women without antibodies statistically the same incidences of the different conditions mentioned above were found all chi square values were less than 3 84 corresponding to p more than 0 05 Only the incidence of stillbirth was statistically significantly higher in the group with CFPAb and LCTAb compared with the control group χ=6 5 0 01<p<0 025 Table 2 shows the mean values of fetal birth weights placental weights and birth weight/placental weight ratio that is fetal birth weight divided by placental weights The mean values were distributed according to antibody content in the women s sera and grouped with reference to the women s smoking habits The mean birth weight was significantly higher in non smokers than in smokers t=9 66 corresponding to p<0 001 with 1987 degrees of freedom The birth weight/placental weight ratio (BW/PW ratio) was also signifi-

omen without CFPAb d LCTAb in serum	
	%
8 of 1 776	0 5
98 of 1 7 6	5 7
122 of 1 715	65 4
584 of 1 715	34 1
9 of 1 715	0 5
17 of 1 679	1 0
22 of 1 679	1 3

cantly higher in non smokers compared with smokers t=5 35 $p<0\ 001$ with 1916 degrees of freedom No statistically significant difference of the mean values of birth weights placental weights or BW/PW ratios were found when cases with antibodies were compared with cases without antibodies and the smoking habits of the women were considered

DISCUSSION

The assays applied in the present investigation may detect immune antibodies of IgM and IgG classes Both types of immunoglobulins are able to fix complement but only IgG is able to enter fetal circulation We did not perform immuno globulin separation studies and therefore the question of immuno-globulin classes of the antibodies in question remains unanswered Payne et al (21) observed no harmful effect of leuco agglutinins in the serum of newborn We found demonstrable autocytotoxic antibodies of maternal origin in two cord blood samples without any symptoms of harmful effect Even though IgM immune antibodies are unable to cross the placenta they might be harmful to the placenta but this did not seem to be so in the case reviewed by Simmons (24)

Maternal antibodies and stillbirth Many injuries may cause fetal death Aho et al (1) suggested a possible association between maternal virus infections and stillbirth Jensen (13) found causative relation between leuco-agglutinins and stillbirth Naito et al (17) and Terasaki et al (26) and later on Ahrons (2) found no association either between LCTAb and stillbirth In the current investigation there seemed to be a tendency to a higher incidence of stillbirth in women with CFPAb and LCTAb but the number of stillbirths was too small to draw definite conclusions

Maternal antibodies and birth defects Innumerable injuries may cause fetal malformations (28) Therefore it is rather difficult to draw final conclusions from investigations concerning only a few parameters Jensen (13) and Payne et al (21) observed no positive association between leuco-agglutinins and congenital defects Naito et al (17) found a statistically significant correlation between LCTAb and birth defects Sever et al (23) Terasaki et al (26) and Ahrons (2) could not confirm these findings and neither could we (18) We found 117 cases with birth defects Three babies had malformations of the digestive tract 3 had retentiotestis 31 had luxatio coxae 5 had defects in the central nervous system 4 had cardiac defects 13 had pes varus and 58 had other minor defects Three women with malformed babies had Rh antibodies but no CFPAb or LCTAb In 2 cases of birth defects other irregular antibodies against erythrocytes were demonstrated without CFPAb or LCTAb No demonstrable association between CFPAb or LCTAb and congenital malformations was found

Maternal antibodies and bilirubinaemia The most severe cases of neonatal jaundice occur in Rh haemolytic disease but other blood group incompatbilities may be the cause of neonatal jaundice as reviewed by Mollison (16) and Tovey et al (27) The physiological neonatal jaundice very infrequency exceeds serum bilirubin values over 200 μmol per litre Only 194 babies had maximum bilirubin levels

Table 2 *Mean values of fetal birthweights placental weights and birth weight/placenta weight ratio*

	Fetal birthweight					Placental weight				
	x	S D	No	Max	Min	*x*	S D	No	Max	Min
Non smokers										
Total	3 501	551	1 162	5 500	600	649	138	1 123	1 300	200
Women with CFPAb without LCTAb in serum	3 519	366	8	3 950	2 800	679	175	7	1 000	450
Women with CFPAb and LCTAb in serum	3 318	693	30	4 300	750	587	140	30	900	250
Women without CFPAb with LCTAb in serum	3 486	514	140	4 650	1 350	639	131	134	1 000	330
Women without CFPAb and LCTAb in serum	3 509	552	984	5 500	600	652	139	952	1 300	200
Smokers										
Total	3 261	543	827	4 900	950	6[illegible]8	1[illegible]6	795	1 100	[illegible]00
Women with CFPAb without LCTAb in serum	3 075	491	6	3 550	2 400	625	151	6	750	350
Women with CFPAb and LCTAb in serum	2 945	697	14	3 900	1 485	589	165	14	850	[illegible]00
Women without CFPAb with LCTAb in serum	3 332	562	81	4 400	1 310	635	141	79	1 050	350
Women without CFPAb and LCTAb in serum	3 260	537	7[illegible]6	4 900	950	628	123	696	1 100	[illegible]00

of more than 200 μmol per litre and in 11 cases the serum bilirubin was higher than 300 μmol Ahrons (2) found no influence of HL A LCTAb on the severity of jaundice of newborns of Rh immunized women We too found no association between LCTAb or CFPAb and severity of neonatal jaundice

Maternal antibodies and neonatal diseases Neonatal neutropenia and thrombocytopenia may occur on account of maternal antibodies We found no cases of neonatal puerpura or leucopenia in our study The percentage of children with postnatal asphyxia was statistically the same in all groups The numbers were too small to draw definite conclusions The incidences of infections in the postnatal period were almost the same in all groups of children CFPAb and LCTAb seemed to have no influence upon neonatal resistance against infections

Maternal antibodies and fetal birth weight Jensen (12 13) observed a significantly higher incidence of birth weights below 2 500 g in women with leucoagglutinins This observation could not be confirmed by Brain (6) Nor could Ahrons (2) find any influence of LCTAb on fetal birth weights We found a significantly higher birth weight in non smokers than in smokers but no association between CFPAb and LCTAb and birth weight

Maternal antibodies and placental weight It has been claimed that feto maternal ABO incompatibility significantly increased the placental weight (25) Placental weight should always be viewed in the light of the corresponding birth weight and therefore the BW/PW ratio was applied The BW/PW ratio was found significantly higher in non smokers than in smokers which means that the placenta is relatively smaller in non smokers but no positive correlation was found between CFPAb or LCTAb and relative placental weight

From the current investigation it could be concluded that CFPAb and LCTAb in pregnant women have no harmful effect upon the fetus even if a direct cross match test is positive *Lalezari & Bernard* (14) suggested that antibodies against histocompatibility antigens

Birth weight/Placental weight				
I	S D	No	Max	Min
5 53	0 97	1 1?3	11 00	2 00
5 58	1 17	7	7 44	3 95
5 80	1 41	30	9 50	3 11
5 60	1 0?	134	9 09	3 00
5 51	0 95	952	11 00	2 00
5 30	0 90	795	10 00	2 11
3 11	1 08	6	6 86	3 64
5 13	1 01	14	7 00	3 50
5 88	0 91	79	7 89	3 1?
?79	0 90	696	10 00	2 11

do not cause harmful conditions in the fetus because they will be absorbed by the placenta and various tissues to such a degree that their effect will be negligible Only leucocyte or thrombocyte specific antibodies may unhindered cross the placenta and cause neutropenia or thrombocytopenia This suggestion was in contrast to the observations published by Colombani et al (9) and the findings in our study Even though we know the *in vitro* effect of the antibodies formed during pregnancy we are still unable to demonstrate their clinical effect as put forward in an earlier paper (19)

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REFERENCES

1 Aho K Cantell K Hjelt L & Rantasalo I Possible association between maternal virus infections and stillbirths *Br J Soc Med 22* 38 1968

2 Ahrons S HL A antibodies Influence on human foetus *Tissue Antigens 1* 1 9 1971

3 Ahrons S & Glavind Kristensen S Cytotoxic HL A antibodies Immunoglobulin classification *Tissue Antigens 1* 121 1971

4 Bast G Leverenz S & Kellner R Nachweis einer diaplazentar übertragenen Immunothrombopenie mit Hilfe der Immunofluoreszenztechnik *Folia Haematol 87* ?37 1967

5 Boxer L A Yokoyama M & Lalezari P Isoimmune neonatal neutropenia *J Pediatr 80* 783 1972

6 Brain P Leucocyte agglutinins and birth weight *Med J Aust 1* 1046 1968

7 Brambell F W R The transmission of immune globulin from the mother to the foetal and newborn young *Proc Nutr Soc 28* 35 1969

8 Ceppellini R Tissue and humoral immunity in foeto-maternal relationships *Proc Int Congr Immunol Obstet Gynaecol* Padua Italy 1973 (in press)

9 Colombani J Colombani M & Dausset J Two cases of neonatal thrombocytopenia due to maternal iso-immunization against leucoplatelet antigens *Vox Sang 14* 137 1968

10 Hill B *Principles of medical statistics* The Lancet Ltd London 8th ed

11 Hitzig W H & Gitzelmann R Transplacental transfer of leucocyte agglutinins *Vox Sang 4* 445 1959

12 Jensen K G Leucocyte antibodies in serum of pregnant women *Vox Sang 7* 454 1962

13 — Leucocyte antibodies in serum of pregnant women Serology and Clinic II *Vox Sang 9* 315 1964

14 Lalezari P & Bernard G E An isologous antigen–antibody reaction with human neutrophiles related to neonatal neutropenia *J Clin Invest 45* 1741 1966

15 McKay E & Thom H Antibodies to gamma globulin in pregnant women Incidence aetiology and size *J Obstet Gynaecol Br Commonw 78* 345 1971

16 Mollison P L *Blood transfusion in clinical medicine* Blackwell Oxford 1967 4th ed

17 Naito S Mickey M R Ebbin A J Strauss J & Terasaki P I Fetal maternal incompatibility II Direct cross match studies on 38 children with birth defects In *Histocompatibility Testing 1970* Munksgaard Copenhagen 1970

18 Nymand G Heron I Jensen K G & Lundsgaard A Cytotoxic antibodies in serum of pregnant women at delivery *Acta Pathol Microbiol Scand B 79* 595 1971

19 Nymand G Complement fixing and lymphocytotoxic antibodies in serum of pregnant women at delivery *Vox Sang 27* 273 1974

20 Overweg J & Engelfriet C P Cytotoxic leucocyte iso-antibodies formed during the first pregnancy *Vox Sang 16* 97 1969

21 Payne R Rolfs M R Tripp M & Weigle J Neonatal neutropenia and leucoagglutinins *Pediatrics 33* 194 1964

22 Pearson H A Shulman N R Marder V J & Cone T E Isoimmune neonatal thrombocytopenic purpura *Blood 23* 154 1964

23 Sever J L & Terasaki P I Maternal fetal incompatibility III Central nervous system and cardiac

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of more than 200 μmol per litre and in 11 cases the serum bilirubin was higher than 300 μmol Ahrons (2) found no influence of HL A LCTAb on the severity of jaundice of newborns of Rh immunized women We too found no association between LCTAb or CFPAb and severity of neonatal jaundice

Maternal antibodies and neonatal diseases Neonatal neutropenia and thrombocytopenia may occur on account of maternal antibodies We found no cases of neonatal puerpura or leucopenia in our study The percentage of children with postnatal asphyxia was statistically the same in all groups The numbers were too small to draw definite conclusions The incidences of infections in the postnatal period were almost the same in all groups of children CFPAb and LCTAb seemed to have no influence upon neonatal resistance against infections

Maternal antibodies and fetal birth weight Jensen (12 13) observed a significantly higher incidence of birth weights below 2 500 g in women with leucoagglutinins This observation could not be confirmed by Brain (6) Nor could Ahrons (2) find any influence of LCTAb on fetal birth weights We found a significantly higher birth weight in non smokers than in smokers but no association between CFPAb and LCTAb and birth weight

Maternal antibodies and placental weight It has been claimed that feto maternal ABO incompatibility significantly increased the placental weight (25) Placental weight should always be viewed in the light of the corresponding birth weight and therefore the BW/PW ratio was applied The BW/PW ratio was found significantly higher in non smokers than in smokers which means that the placenta is relatively smaller in non smokers but no positive correlation was found between CFPAb or LCTAb and relative placental weight

From the current investigation it could be concluded that CFPAb and LCTAb in pregnant women have no harmful effect upon the fetus even if a direct cross match test is positive Lalezari & Bernard (14) suggested that antibodies against histocompatibility antigens

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THE USE OF CLINICAL GESTATIONAL AGE ASSESSMENT IN THE CONSTRUCTION OF STANDARDS FOR BIRTHWEIGHT IN A RURAL NIGERIAN COMMUNITY

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ABSTRACT Brueton M J (Department of Paediatrics Ahmadu Bello University Teaching Hospital Zaria Nigeria) The use of clinical gestational age assessment in the construction of standards for birthweight in a rural Nigerian community Acta Paediatr Scand, 64 537 1975.—The management of low birthweight infants in the developing world is complicated by the lack of criteria for distinguishing between preterm and small for gestational age newborns Clinical gestational age assessment has been applied to 850 normal singleton neonates in a rural Nigerian community The technique used was sufficiently accurate to enable centile curves for birthweight and gestational age to be constructed Comparison with a European community showed that in the African babies weight gain was reduced in late pregnancy and had ceased by 40 weeks gestation but that the median birthweight was less at each week of gestation studied The preparation and use of such reference curves for birthweight and gestational age for the study of the reasons for these differences is discussed

KEY WORDS Gestational age assessment low birthweight African neonates

Although the proportion of low birthweight (BW) infants born in the developing world is much greater than in America or Western Europe criteria for the distinction between preterm and small for gestational age (GA) babies are unstandardised and so statistics relating to the perinatal period are difficult to interpret (8) Furthermore the construction of intrauterine growth curves has been restricted to selected and relatively affluent communities in which mothers have known the date of their last menstrual period (2 9) This method is unrealistic in the large areas of the world where most mothers are unable to provide this information In a preliminary study of 50 neonates however it was shown that a scoring system for GA assessment using the neurological and external characteristics described by Dubowitz et al (4) could be applied satisfactorily to a rural Nigerian community (1) This study was therefore planned to apply the method to a large number of Nigerian babies so that standard curves of BW for GA could be constructed

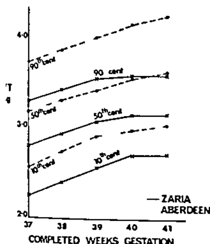
METHODS

The study was carried out in the obstetric unit of Ahmadu Bello University Hospital Zaria which is situated in the savana belt of West Africa The economy of the population concerned is based on agriculture while the hospital services involved were the only medical facilities available in the area All singleton liveborn infants seen between January and May 1972 who were suitable for GA assessment were personally examined within 24 hours of delivery up to one hour prior to their next feed Those suffering from cerebral birth trauma or congenital malformations or who were born of diabetic mothers were excluded from the study The babies were weighed at birth by staff midwives using infant scales which were regularly checked for zero adjustment It was not possible to obtain accurate details of birthrank maternal size or age The distribution of B W s for each completed week of gestation were reviewed for each sex and mean BW and standard devia

anomalies In *Histocompatibility Testing 1970* Munksgaard Copenhagen 1970
24 Simmons R L Histocompatibility and the survival of the fetus Current controversies *Transplant Proc 1* 47 1969
25 Tatra G Muller Settele A Wolf E & Placheta P Placentagewicht und feto maternale Inkompatibilitat *Z Geburtshilfe Gynaekol 176* 464 1972
26 Terasaki P I Mickey M R Yamazaki J N & Vredevoe D Maternal fetal incompatibility *Transplantation 9* 538 1970
27 Tovey L A D Taverner J M & Longster G H The effect of environment on ABO antibodies *Vox Sang 19* 64 1970
28 Tuli S M Certain causes of congenital malformations in homo sapiens and their prevention *J Indian Med Assoc 49* 527 1967
29 White D Haidar A & Reinhold J G Spectophotometric measurement of bilirubin concentrations serum of newborn *Clin Chem 4* 211 1958
30 Williams R C Emmons J D & Ynis E J Studi of human sera with cytotoxic activity *J Clin Inves 50* 1514 1971

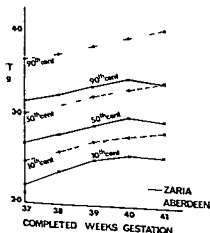
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g 2 Birthweight and gestational age curves for single n male infants born in Zaria compared with those born Aberdeen

The introduction of standards for the rec gnition of small for GA and preterm infants in opulations where there is a high incidence of w BW newborns and a high perinatal mortal y is useful in three main respects Firstly they low comparison with similar studies in com unities with different cultural and economic ackgrounds and secondly they may be used a standard of reference for subsequent udies within the areas concerned of the ef cts of such measures as malaria control (6) nd improved antenatal care Gruenwald (7)



g 3 Birthweight and gestational age curves for single n female infants born in Zaria compared with those orn in Aberdeen

noted that as the socioeconomic status of a community improved so the mean weight for GA of the foetus increased and foetal growth rates were maintained to a later stage of preg nancy however the exact roles of the indi vidual maternal foetal and environmental factors involved have proved difficult to define (3 10) In order to investigate these problems further it is important to characterise the in fant population more accurately than by BW since it is now accepted that this alone is an inadequate parameter The presentation of BW for GA curves in the population involved is thus an essential preliminary step The third important use of such standards is in the clini cal care of the newborn so that the problems of management of different groups of low BW infants in the developing world can be more clearly defined and the allocation of resources in the delivery of perinatal care planned ac cordingly

ACKNOWLEDGEMENTS

I am indebted to the nursing staff of Ahmadu Bello Uni versity Obstetric Unit for their co-operation during this project and to Dr H Lejarraga for statistical advice

REFERENCES

1 Brueton M J Palit A & Prosser R Gestational age assessment in Nigerian newborn infants *Arch Dis Child 48* 318 1973
2 Cheng M C E Chew P C T & Ratnam S S Birthweight distribution of Singapore Chinese Malay and Indian infants from 34 weeks to 42 weeks gesta tion *J Obstet Gynaecol Br Common* 79 149 1972
3 Donnelly J M Flowers C E Creadick R N Wells H B Greenberg B G & Surles K B Maternal foetal and environmental factors in pre maturity *Am J Obstet Gynecol 88* 918 1964
4 Dubowitz C Dubowitz V & Goldberg C Clinical assessment of gestational age in the newborn infant *J Ped atr 77* 1 1970
5 Finnstrom O Studies on maturity in newborn in fants II External characteristics *Acta Paediatr Scand 61* 74 1972
6 Gilles H M Lawson J B Sibellas M Voller A & Allan N Malaria anaemia and pregnancy *Ann Trop Med Parasitol 63* 45 1969
7 Gruenwald P Fetal growth as an indicator of socioeconomic change *Public Health Rep 83* 867 1968

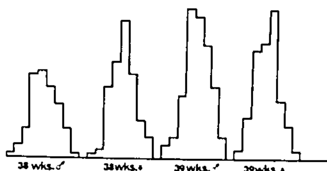


Fig 1 Examples of the distribution of birthweights for single weeks of gestation Each vertical column represents a 250 g group

tions determined Centile figures were calculated from the latter results and curves for BW and GA drawn and smoothed by eye Values for BW to the nearest 10 grams for each week of gestation were derived from these

RESULTS

850 infants of 37 to 41 weeks gestation were examined The number of singleton deliveries seen below 37 weeks gestation were too few for statistical analysis Fig 1 shows that there is a normal distribution of BW for each completed week of gestation near term Table I shows the median standard deviation calculated centile and smoothed mean and centile values for BW for each sex and week of gestation The smoothed figures are very similar to the calculated ones The boys were heavier than the girls at all ages and both ceased to gain weight after 40 weeks gestation Figs 2 and 3 show the BW s for GA graphically and compare the results with those found amongst the infants of multiparous mothers in Aberdeen (12) The Nigerian babies were lighter at all ages and there was an earlier cessation of intrauterine weight gain

DISCUSSION

The technique of GA assessment used here produced consistent results there was a normal distribution of BW s and the original mean and centile curves obtained required very little smoothing It seems probable therefore that the results are a fairly accurate reflection of the intrauterine growth pattern It is not however possible from this study to make further observations on the reliability of the assessment technique regarding its application to African infants (11) or to infants of low BW It has been observed that some external characteristics tend to overestimate GA at low score values while conversely they may underestimate in small for GA infants compared with appropriate for GA infants of the same maturity (5) It was decided on the basis of the previous work carried out in this community (1) that the regression line proposed by Dubowitz (4) was valid and did not need to be restandardised

Table 1 *Median birthweights centile and standard deviation by sex and gestational age*

						Smoothed		
Gestation (completed weeks)	No of cases	Mean BW (g)	Stnd Dev (g)	10th centile (g)	90th centile (g)	Mean BW (g)	10th centile (g)	90th centile (g)
Male								
37	25	2 800	453	2 220	3 380	2 800	2 300	3 300
38	91	2 900	382	2 411	3 389	2 940	2 400	3 440
39	143	3 080	399	2 569	3 591	3 080	2 570	3 560
40	142	3 150	330	2 727	3 573	3 150	2 720	2 580
41	52	3 150	344	2 709	3 591	3 150	2 715	3 590
Female								
37	31	2 714	476	2 168	3 260	2 700	2 210	3 180
38	109	2 820	353	2 368	3 272	2 830	2 370	3 [illegible]80
39	129	2 960	428	2 532	3 388	2 960	2 530	3 390
40	97	3 050	355	2 595	3 505	3 050	2 600	3 500
41	31	3 010	341	2 573	3 447	3 010	2 580	3 450

Acta Pædiatr Scand 64 541–545 1975

HOSPITAL CROSS INFECTION ON CHILDREN S WARDS WITH RESPIRATORY SYNCYTIAL VIRUS AND THE ROLE OF ADULT CARRIAGE

D G SIMS M A P S DOWNHAM J K G WEBB
P S GARDNER and DORIS WEIGHTMAN

From the Departments of Child Health and Virology Royal Victoria Infirmary and the Department of Medical Statistics University of Newcastle upon Tyne Newcastle upon Tyne England

ABSTRACT Sims D G Downham M A P S Webb J K G Gardner P S and Weightman D (Departments of Child Health and Virology Royal Victoria Infirmary and Department of Medical Statistics University of Newcastle upon Tyne England) Hospital cross-infection in children s wards with respiratory syncytial virus and the role of adult carriage Acta Paediatr Scand 64 541 1975 —A further 18 children who acquired respiratory syncytial (R.S) virus infection on general paediatric wards have been identified bringing the total number of hospital cross-infections by this virus to 46 for the last 3 annual R S virus epidemics on Tyneside 25 of these 46 illnesses involved the lower respiratory tract Carriage of R S virus by staff and visitors was studied on one ward and appeared to be responsible for at least 2 and probably 4 of the 6 cross-infections detected on this ward Methods for controlling the transmission of virus by adults on children s wards are discussed

KEY WORDS Respiratory syncytial virus children cross-infection

The frequency and severity of illnesses acquired by children in hospitals and nurseries as a result of cross infection with respiratory viruses has been the subject of several recent reports (1 2 3) Cross infection rates for wards of differing design have indicated that the use of individual cubicles reduces but does not eliminate the risk of cross infection Carriage by staff and visitors must therefore be a factor in the cross infection process This paper describes the cross infections detected in 6 Tyneside hospitals during a further respiratory syncytial virus (R S virus) epidemic and the part played by staff and visitors in the transmission of infection from child to child Possible ways of controlling this factor are discussed

METHODS

Detection of c oss infection illnesses

A total surveillance of all children admitted with acute respiratory illness to the hospitals in this study is maintained specimens for virus diagnosis being taken on admission Specimens are also taken from any child who develops symptoms of a respiratory illness while in hospital The methods of rapid virus identification virus isolation and the clinical categorisation of the illnesses have been described in full elsewhere (4 5)

A cross infection illness was defined as any illness associated with the identification of R S virus in which the onset of symptoms occurred more than 5 days after admission to hospital Although a slightly shorter incubation period (3–5 days) has been estimated for R S virus on the basis of challenge experiments in adult volunteers (6) this artificial model may not necessarily hold true for natural infection in children who in our experience incubate the virus for between 5 and 8 days

Cross-infection rates for different wards were calculated and compared by the methods described by Weightman et al (7) This gives a measure of the frequency of cross infection in different wards taking into account the number of and length of stay of R S virus infected children and of those at risk of cross-infection Cross infection rate is defined as follows

Cross infection rate=

Number of cross infections per million susceptibles per infective day=

$$\frac{\text{No of cross-infections} \times 10 \times \text{length of study}^1}{(\text{No at risk} \times \text{mean stay}) \times (\text{No infected} \times \text{mean stay})}$$

Detection of adult carriers

Adult carriage was studied in one ward only selected because it is made up mainly of individual cubicles and was

[1] Length of study=days from admission of first child with R.S virus infection to a ward to discharge of last child with R S virus infe tion from that ward

8 Jansen A A Birthweight birthlength prematurity and neonatal mortality in New Guineans *Trop Geogr Med 14* 341 1962
9 Malan A F Evans A De V Smit W B & De V Heese H Intrauterine growth a study of the birth weights of liveborn infants *S Afr Med J 41* 698 1967
10 Naeye R L Diener M M Harcke H T & Blanc W A Relation of poverty and race to birthweight and organ and cell structure in the newborn *Pediatr Res 5* 17 1971
11 Parkin J M The assessment of gestational age in Ugandan and British newborn babies *Dev Med Child Neurol 13* 784 1971
12 Thomson A M Billewicz W Z & Hytten F E The assessment of fetal growth *J Obstet Gynaecol Br Commonw 75* 903 1968

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Table 1 *The ages of the 18 children with R S virus hospital cross infection and the illnesses acquired*

A and B indicate wards (see text) U R T I =Upper respiratory tract infection

Age	Acquired illness
2 months	Bronchiolitis
2 months	Bronchiolitis[A]
3 months	Bronchitis[A]
4 months	Bronchiolitis[B]
4 months	Bronchiolitis[B]
7 months	Pyrexia and vomiting[A]
8 months	Bronchiolitis[B]
9 months	Croup
12 months	Pneumonia[A]
12 months	U R T I [B]
14 months	U R T I [A]
16 months	Bronchitis
23 months	U R T I
2 years	U R T I [B]
2 years	U R T I [B]
3 years	Bronchitis[A]
3 years	U R T I [B]
6 years	Bronchitis

expected to receive the largest share of respiratory admissions to the University Hospital Group at the time of the study The ward contains 18 individual cubicles 9 of them with a bed for the mother and a section capable of taking 4 cots together used only for children over one year old There is also a play nursery used by children over a year who are well enough to be out of bed during the day time Children over the age of 5 years are not admitted to the ward

All staff in contact with the children including doctors nurses students physiotherapists and domestic staff were asked to record daily the presence or absence of respiratory symptoms however slight on a chart displayed on a notice board Parents of children admitted were given a written notice at the time of admission asking them to report to sister any respiratory symptoms in themselves or in others visiting their children

Whenever the presence of respiratory symptoms was reported a cough swab was taken on the first day of the symptoms regardless of the degree of severity At the same time a description of the symptoms was obtained Isolation of R S virus from the cough swabs was attempted by tissue culture methods previously described Cough swabs were taken only from those with respiratory symptoms in view of studies which have demonstrated the extreme rarity of asymptomatic carriage of R S virus in adults (8)

A daily record was kept by the nursing staff of the cot number of each child and of all staff visitors or other children in contact with him

Staff were instructed to continue to exercise their individual discretion about whether or not to take sick leave at the time of symptoms this being the usual practice of the ward

RESULTS

Cross infection on all wards

Between December 1972 and April 1973 316 children were admitted to six Tyneside hospitals as a result of R S virus infection During the same period 18 illnesses due to hospital cross infection by R S virus were detected

Table 1 shows the nature of the illnesses acquired by cross infection and the ages of the affected children

Cross infection rates were calculated for the two wards (A and B) which received the largest number of children (96) with R S virus infection Rates were not calculated for the other wards because surveillance for cross infection was less complete in these wards Wards A and B are of contrasting design with regard to their content of individual cubicles Ward A described above has approximately 80% of its cots as individual cubicles while ward B consists of 4 small sections each containing 4 cots together with only 4 individual cubicles Despite the contrast in the design of these two wards the difference between their cross infection rates shown in Table 2 does not reach statistical significance ($z=1\ 15$)

The role of adult carriage on ward A

In this ward 51 children were admitted during the study period because of R S virus infection and 6 cross infections were detected giving a cross infection rate of 1 205 per million susceptibles per infective day

129 cough swabs were taken from staff and parents at the time of respiratory symptoms

Table 2 *R S virus cross infection in 2 wards of contrasting design*

	Percentage individual cubicles	No of R S virus admissions	No of R S virus cross infections	Cross infection rate
Ward A	80	51	6	1 205
Ward B	20	45	7	2 61
				=1 15

Table 3 *Results of cough swabs taken from 129 ward staff and parents at the time of respiratory symptoms*

	R S virus positive	R S virus negative
Nurses	2	63
Doctors	1	15
Mothers	5	22
Fathers	–	2
Domestic staff	–	14
Physiotherapists	–	3
Virology graduate	1	–
Medical student	–	1
Total	9	120

From 9 of these R S virus was identified and Table 3 shows the occupations of those with virus positive and with virus negative swabs 5 out of the 51 mothers of children admitted with R S virus infection were identified as carriers 24 were asymptomatic and therefore not investigated

The R S virus positive illnesses in the adults were all mild upper respiratory tract infections the worst being described as a bad sore throat It was not possible to distinguish these illnesses clinically from the large bulk of R S virus negative illnesses

Of the 6 cross infections detected 2 were likely to have been the result of direct transmission from child to child In both instances the cross infected child had been in contact with an R S virus infected child in the play nursery and in 1 of the 2 there was also contact with infection in the four bedded section

In 4 of the 6 cases there was no such direct contact the children having been nursed in individual cubicles throughout their admission In 2 of these 4 instances the child was known to have been in contact at the appropriate time with an adult excreting R S virus in one instance a mother of another child in the unit and in the other a nurse In the remaining 2 instances no adult carrier was detected These findings are summarised in Table 4 Of the 4 illnesses apparently transmitted by adults 3 involved the lower respiratory tract

DISCUSSION

The demonstration that 18 children acquired R S virus illnesses while in hospital 11 with lower respiratory tract involvement further emphasises that cross infection with this agent in general children s wards is a significant problem A total of 46 R S virus hospital cross infections has now been detected during the past 3 epidemics on Tyneside (2 3)

Of these 46 children 26 were under one year of age and in 17 the cross infection resulted in clinical bronchiolitis These figures may have implications for the incidence and nature of R S virus illnesses in the community as a whole but as it is possible that some mild cross infection illnesses were not detected in this study no firm conclusions can be drawn Even in a unit specially alerted to the risk of cross infection and aware that R S virus may be excreted for up to 14 days from the onset of illness (9) 6 cases occurred during this single epidemic It seems not unreasonable to assume that in children s wards where staff are less aware of the risks and do not have the facilities for rapid virus diagnosis which permits segregation of children with different virus infections the size of the problem may be even greater

The cross infection rate for the unit consisting mainly of individual cubicles was lower than that for a unit poorly supplied with cubicles But the finding that the difference between these two rates did not reach statistical significance is compatible with the existence of an element of adult carriage in the process of cross infection with R S virus

Adult carriage by staff or visitors seems to have been responsible for at least 2 and proba

Table 4 *The part played by adult carriage in 6 R S virus hospital cross infections*

Child to child	2
Adult carriage identified	2
Adult carriage probable but not identified	2
Total cross infections detected	6

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DISCUSSION

The demonstration that 18 children acquired R S virus illnesses while in hospital 11 with lower respiratory tract involvement further emphasises that cross infection with this agent in general children s wards is a significant problem A total of 46 R S virus hospital cross infections has now been detected during the past 3 epidemics on Tyneside (2 3)

Of these 46 children 26 were under one year of age and in 17 the cross infection resulted in clinical bronchiolitis These figures may have implications for the incidence and nature of R S virus illnesses in the community as a whole but as it is possible that some mild cross infection illnesses were not detected in this study no firm conclusions can be drawn Even in a unit specially alerted to the risk of cross infection and aware that R S virus may be excreted for up to 14 days from the onset of illness (9) 6 cases occurred during this single epidemic It seems not unreasonable to assume that in children s wards where staff are less aware of the risks and do not have the facilities for rapid virus diagnosis which permits segregation of children with different virus infections the size of the problem may be even greater

The cross infection rate for the unit consisting mainly of individual cubicles was lower than that for a unit poorly supplied with cubicles But the finding that the difference between these two rates did not reach statistical significance is compatible with the existence of an element of adult carriage in the process of cross infection with R S virus

Adult carriage by staff or visitors seems to have been responsible for at least 2 and proba

Table 4 *The part played by adult carriage in 6 R S virus hospital cross infections*

Child to child	2
Adult carriage identified	2
Adult carriage probable but not identified	2
Total cross-infections detected	6

bly 4 of the 6 cross infections where this factor was studied, and the resulting illness was severe in 3 of the 4 cases The frequency of infection in potential adult carriers may well have been underestimated in this study as cough swabs are known to be approximately 15% less sensitive than naso pharyngeal secretions for the isolation of R S virus (10) Unfortunately the illnesses in the carriers were not different in type or severity from many mild respiratory illnesses not apparently associated with R S virus in staff and visitors Furthermore the large number of these R S virus negative illnesses in adults even during an epidemic period would make an attempt to identify carriers by rapid virus diagnosis impracticable

In the absence of any practical method of identifying carriers either clinically or virologically, what can be done to try to reduce the risk of this mechanism of cross infection? It certainly seems out of the question to exclude all those with mild respiratory symptoms from the care of children in hospitals, although no attempt was made in this study to identify the transmission of other virus types However a wider awareness of the risks at epidemic periods should make it possible to delegate the more intimate aspects of care such as feeding to staff without respiratory symptoms especially for children aged under one year which is the time when R S virus is most likely to produce severe illness The paediatrician has a responsibility to keep a careful surveillance of respiratory infections in all ward staff at these times, and to make appropriate recommendations about their movements rather than rely on individual discretion Close relatives of children admitted with R S virus infection often have the same infection simultaneously (11) In this study 10% of the mothers of children admitted with R S virus infection were identified as carriers so that it would seem wise to warn these mothers to avoid contact with any other children in the ward

It becomes increasingly apparent that children with acute respiratory infections would be best admitted to wards designed for the purpose, where specially interested staff and rapid virus diagnosis could combine to reduce the number of these iatrogenic illnesses

ACKNOWLEDGEMENTS

This study was made possible by the patient cooperation of staff and parents on ward A in reporting their symptoms and allowing us to take cough swabs

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REFERENCES

1 Sterner G Wolontis S Bloth B & De Hevesy G Respiratory syncytial virus An outbreak of acute respiratory illness in a home for infants *Acta Paediatr Scand 55* 273 1966
2 Ditchburn R K McQuillin J Gardner P S & Court S D M Respiratory syncytial virus in hospital cross infection *Br Med J III* 671 1971
3 Gardner P S Court S D M Brocklebank, J T Downham M A P S & Weightman D Virus cross infection in paediatric wards *Br Med J II* 571 1973
4 Gardner P S & McQuillin J Application of immunofluorescent antibody technique in rapid diagnosis of respiratory syncytial virus infection *Br Med J III* 340 1968
5 Gardner P S Stanfield J P Wright A E Court S D M & Green C A Viruses bacteria and respiratory disease in children *Br Med J I* 1077 1960
6 Kravetz H M Knight V Chanock R M Morris J A Johnson K M Rifkind D & Utz J P Respiratory syncytial virus III Production of illness and clinical observations in adult volunteers *JAMA 176* 657 1961
7 Weightman D Downham M A P S & Gardner P S Introduction of a cross infection rate in children s wards and its application to respiratory virus infections *J Hyg (Camb) 73* 53 1974
8 Chanock R M Kim H W Vargosko A J Deleva A Johnson K M Cumming C & Parrott R H Respiratory syncytial virus I Virus recovery and other observations during 1960 outbreak of bronchiolitis pneumonia and minor respiratory diseases in children *JAMA 176* 647 1961
9 Gardner P S McQuillin J & McGuckin R The late detection of respiratory syncytial virus in cells of respiratory tract by immunofluorescence *J Hyg (Camb) 68* 575 1970
10 Sturdy P M McQuillin J & Gardner P S A

comparative study of methods for the diagnosis of respiratory virus infections in childhood *J Hyg (Camb)* 67 659 1969
11 Berglund B Respiratory syncytial virus infections in families A study of family members of children hospitalised for acute respiratory disease *Acta Paediatr Scand* 56 395 1967

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CASE REPORT

INJURY OF THE SPINAL CORD AT BIRTH

A Report of Two Cases

U LINDBERG B HAGBERG Y OLSSON and P SOURANDER

From the Department of Paediatrics II and Institute of Pathology University of Goteborg and Institute of Pathology University of Uppsala Sweden

ABSTRACT Lindberg, U, Hagberg B Olsson Y and Sourander P (Department of Paediatrics II and Institute of Pathology, University of Goteborg and Institute of Pathology University of Uppsala Sweden) Injury of the spinal cord at birth A report of two cases Acta Paediatr Scand 64 546 1975 —Spinal cord injury may occur as a severe complication to delivery In the vast majority of such cases the injury results from a traumatic breech delivery but cases of injuries after cephalic presentation and fetal malposition have also been described Two cases are reported One of the infants died at the age of 8 months and neuropathological examination of the brain and spinal cord was performed The other child now 6 years old is still alive Incidence mechanism of injury clinical and morphological features and treatment are briefly discussed

KEY WORDS Birth injury spinal cord palsy

A century ago Parrot described the clinical and pathological findings in an infant with cervical cord injury with normal spinal reflexes and quadriplegia (15) During the difficult breech delivery un craquement tres fort was heard and at autopsy ruptures and hemorrhages were seen in the lower cervical cord and the covering meninges

The classical articles on the clinical aspects of intrapartum spinal cord injury were published by Crothers Ford and Putnam (3 4 6) calling attention to the fact that the vast majority of cases follow traumatic breech delivery The clinical features of 28 patients and autopsy findings in 2 cases were described Later clinical and pathological case reports have appeared (9 10 12, 18 20 21) Autopsy studies were performed in several of the reported cases these being mostly infants who died during or soon after delivery (2 8, 16, 19 20)

It is worth emphasizing that spinal cord lesions may still occur as a result of delivery and that they are not merely of historical interest

CASE REPORTS

Case 1

L J This girl the third child of a healthy 28 year-old woman was born after 40 weeks of gestation The pregnancy was uneventful The onset of labour was spontaneous with foot presentation The arms were extended and could be delivered only with difficulty However the head followed without any problems The birth weight was 3 250 g and the length 52 cm The Apgar score was 3 at 1 min 5 at 3 min and 8 at 10 min The child was ventilated and regained normal colour and maintained a normal cardiac rate She was placed in an incubator containing 28% oxygen

On the second day there were brief episodes of increased tone in the limbs and the child was inactive On the fourth *day there were no spontaneous movements of the legs*

Supported by grants from the Swedish Medical Research Council Project B74 12X 3020-05C



Fig 1 Cross section from the spinal cord in the atrophic segment The normal parenchyma is replaced by glial cell and connective tissue cells rich in blood vessels Case 1

X-ray examination of the vertebral column was normal At one month it was possible to nurse the baby at home

At the age of 3 months the child appeared to have reached an average mental development she smiled followed and fixed with her eyes The motor activity in her arms was normal for the age but there were only few and weak spontaneous movements of the legs The respiration was exclusively diaphragmatic and her cry was weak Neurological examination re ealed predominantly flaccid pareses in the lower limbs There were obvious signs of decreased reaction to painful stimuli up to a level just above the nipples No significant tendon reflex responses could be elicited from the legs A spinal cord lesion of unknown etiology at the level of T 1 was presumed

At 4 months of age the girl suffered from an upper respiratory infection followed by respiratory insufficiency For a short time she was placed in a ventilator To exclude a spinal cord tumour an oxygen myelography was performed No gas spread above the level of T 3 Since an expansive process could not be excluded laminectomy was performed No tumour was found but the spinal cord was atrophic and adherent to the dura at T 2–T 3 Efforts were also made to release the cord from the dura

At the age of 6 months the girl developed another re spiratory infection with pulmonary insufficiency and was again placed in a ventilator She had combined flaccid and spastic pareses in her legs with spinal automatism bladder and rectum pareses and impaired sensibility to pain below the level of T 1 At the age of 8 months the girl died from bron hopneumonia

Autopsy

A detailed dissection and histopathological investigation of the central nervous system was performed Multiple specimens from the brain and spinal cord were embedded in paraffin and stained with hematoxylin–eosin and hematoxyl n–van Gieson Other sections were stained with luxol fast blue and cresyl violet for myelin and Nissl bodies with Palmgren s silver technique for axons and a cording to Rar ke for astro-glial cells Finally other sections were stained in iron with Prussian blue to reveal old haemorrhages

At the site of the thoracic laminectomy there were signs of normal healing in the surrounding soft tissues The spinal canal was normal in configuration and the dura did not show any changes The epidural adipose tissue had a brownish tint indicating an old haemorrhage Histology also disclosed haemosiderin when stained with Prussian blue The spinal cord was atrophic for a 0 5 cm long segment in the upper thoracic region the diameters being only about one third of the expected size

Histological examination of the atrophic cord segment showed an almost complete destruction of the tissue (Fig 1) The normal architecture was disturbed and the central part of the cord was replaced by collagen and fibrillar astrocytes In the atrophic region nerve roots (Fig 2) were surrounded by scar tissue and there appeared to be some loss of myelinated fibres The vascularity of the cord surface was reduced 0 5 cm above and below the atrophic parts The vascularity of the remaining part of the cord seemed to be normal In the ascending tracts of the upper cervical region (particularly medial dorsal columns) and in the descending pathways of the lumbar region (*ventral and* lateral columns) there were signs of fibre tracts degenera tion This was interpreted as *Wallerian degeneration* sec ondary to the destroyed area in the thoracic segments

The brain was swollen with a weight of 9 0 g (normal weight about 700 g) (19) The cerebellum had a normal configuration except for slight atrophic changes in the vermis *The cerebral cortex* was of normal thickness On the left side the Ammon s horn and the hippocampal gyrus were paler and firmer than on the right side Histology showed *anoxic is hemic* changes with loss of neurons in different parts of the neopallial cortex and in the left hippocampus The cause of death was tracheobronchitis and bronchopneumonia

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fetal and neonatal autopsies where complete neuropathological examination was performed spinal cord or brain stem injury was observed in 10–33% of the cases (2 20 21 22) while fractures of the spinal column were found in 1% (14)

There are only a few cases surviving a severe injury Compared with 429 children with cerebral palsy born 1959–1968 (7) only one girl with spinal cord injury was diagnosed (Case No 2) Perhaps mild forms exist in the heterogeneous group called minimal brain dysfunction especially within the clumsy child syndrome subgroup

Most probably a relationship exists between the traction stress applied at delivery and the occurrence of spinal cord lesions The vertebral columns of the neonate is poorly ossified and is less rigid than in the adult Since the spinal cord is less elastic than its encasement (18) injuries may occur as a result of traction stress Some aspects of the tensile strength of the spinal column were revealed in Duncan s (5) experiments He applied a traction force of 90 pounds to the vertebral column of stillborn children and found that the column yielded Decapitation occurred at a force of 120 pounds Forceful longitudinal traction during delivery particularly when combined with flexion and torsion of the vertebral axis is thought to be the most important cause of this type of injury (4 16) Since the spinal cord is firmly anchored by the brachial plexus but is only loosely attached to the dura lower cervical and/or upper thoracic lesions are prone to occur in breech presentation (6 12 18 21)

The mechanism of injury during cephalic delivery is more difficult to explain but is probably a result of torsion (17) The site of this lesion is nearly always superior to the brachial attachment (17) Foetal malposition with hyperextension of the head so-called flying fetus may make the child vulnerable to unphysiological force and there are also such cases reported with signs of spinal cord palsy (1 9 11)

Most investigators are of the opinion that the spinal cord lesions are due to direct mechanical trauma but if the spinal cord arteries are also damaged this may result in local ischemia with secondary oedema and hemorrhages aggravating the initial injury (13) Occasionally even the vertebral arteries are damaged (23)

Towbin divided the cases as they present themselves to the clinician into three major groups (21) In the *first* sudden death occurs during delivery or soon thereafter as a result of intolerable stress and injury to vital structures in the brain stem and upper spinal cord during the descent and extraction of the foetus The *second* group comprises cases in which the infants survive for a short period of time The most significant manifestation in this group is depression of respiration complicated by bronchopneumonia The neurological symptoms of spinal shock are associated with limpness In the *third* group the infant survives with a transient or permanent paralysis paraplegic or tetraplegic

The two children described in this paper obviously belong to the third group The first girl was paraplegic and she lay in the characteristic frog position seen in these patients She had a sensory impairment spinal automatism and pareses of the bladder As a result of intercostal muscle pareses she developed respiratory abnormalities and died of bronchopneumonia It is worth noting that the oxygen myelography showed a picture resembling that of an expansive process (9 18) The second patient is tetraplegic with flaccid pareses in the arms She has had several episodes of bronchopneumonia urinary tract infections and femur fractures as complications of the spinal cord injury

The neuropathological features of spinal cord injury at birth have been described in detail by Towbin (21 22) The lesions may consist of laceration oedema congestion acute neuronal damage haemorrhage and necrosis The dura and spinal nerve roots in the damaged segments may be torn Epidural subdural or subarachnoid haemorrhages are often seen



Fig 2 The spinal roots are partly included in the scar tissue (below to the right) Two root fascicles (upper left) are better preserved Case 1

Case 2

U S This girl the first child of a healthy 26-year-old woman was born after 40 weeks of gestation by breech delivery An X ray examination before delivery showed breech position of the child and a normal pelvic configuration of the mother The amniotic fluid was stained with meconium and the umbilical cord was wrapped once round the neck of the child The birth weight was 3 840 g and the length 51 cm It was claimed that no excessive external traction was used at delivery Immediately after birth it was observed that the child was flaccid and had diaphragmatic type of breathing

The child was brought to the paediatric clinic at the age of one week Flaccid pareses were found in the legs in the intercostal muscles and to a lesser extent in the upper limbs The knee reflexes could be elicited Infantile spinal muscular atrophy of the Werdnig Hoffmann type was suspected Electromyography of the right anterior tibial muscle and electroencephalography showed no abnormality The serum levels of lactic dehydrogenase glutamate oxalacetate transaminase and creatine phosphokinase were normal A biopsy from the right quadriceps muscle was unrevealing The cerebrospinal fluid had 154 red and 9 white blood cells/mm^3 the protein content being 180 mg per 100 ml

When the girl was 3 years old an oxygen myelography showed a flattened spinal cord adherent to the dorsal wall of the spinal canal in the lower cervical region The X ray suggested an atrophic process secondary to a traumatic injury Electromyography revealed no signs of denervation in the quadriceps muscles or in the anterior tibial muscles

At 6 years of age there was no clinical change The child was tetraparetic with combined spastic and flaccid pareses in her legs In addition flaccid pareses were present in her arms and the thenar and hypothenar eminences were atrophic She was able to sit but had an extreme kyphoscoliosis (Fig 3) She had a normal mental age Over the years she had had bronchopneumonia four times urinary tract infections seven times and once fractures of the femur

DISCUSSION

Since autopsies of infants do not routinely include an examination of the spinal cord there may be undiscovered cases of spinal palsy caused by birth injury In unselected series of



Fig 3 Case No 2 at six years of age She is tetraparetic is able to sit but has an extreme kyphoscoliosis

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CASE REPORT

INFANTILE SUB LOBAR EMPHYSEMA AND TRACHEAL BRONCHUS

T IANCU Y BOYANOVER N EILAM E ELIAN and M A LERNER

From the Departments of Paediatrics and Radiodiagnosis Sharon Hospital Petach Tikvah and Tel Aviv University Medical School Israel

ABSTRACT Iancu T Boyanover Y Eilam N Elian E and Lerner M A (Departments of Paediatrics and Radiodiagnosis Sharon Hospital Petach Tikvah and Tel Aviv University Medical School Israel) Infantile sub lobar emphysema and tracheal bronchus Acta Paediatr Scand 64 551 1975.—Localised disturbance of aeration in children is as often the result of mucosal swelling due to infection as resulting from an inhaled foreign body The present report adds another cause tracheal bronchus Elucidation of the cause of localised disturbance of aeration usually includes bronchoscopy the anesthesia and direct trauma involved being highly undesirable if infection is the cause The case for limited careful bronchography before or instead of bronchoscopy when the history of foreign body is lacking, is presented

KEY WORDS Tracheal bronchus infantile lobar emphysema mediastinal shift paediatric bronchography

Atelectasis and obstructive emphysema separately or together from occlusive effect of exudate and mucosal swelling frequently complicate acute respiratory infection (3) and may persist after clinical recovery If the mode of onset and the clinical features are not consistent with simple infection aspirated foreign body has to be excluded in infants absence of an eye witness not withstanding The routine radiological examination including the mediastinal shift cannot distinguish between these two etiologies nor exclude rare tracheobronchial anomalies Bronchoscopy despite its being an additional injury to a diseased airway is therefore often resorted to and is as often negative

In a case of localised emphysema here reported bronchography obviated the need for bronchoscopy by revealing a tracheal bronchus considered the causative lesion

CASE REPORT

A 7 month-old male baby developed a mild respiratory tract infection he was feverish for about 4 days and during this period he coughed and moist rales as well as crepitations were heard over the left lung he was given oral ampicillin and the fever and symptoms subsided Three weeks later the symptoms and fever recurred and roentgenographic examination of the chest was performed The AP film (Fig 1a) disclosed shift of the mediastinum to the left and increased transradiancy of the right lung especially of its upper half No atelectatic areas were seen in the left lung The infant was referred to hospital for further examination He was a healthy looking child by now 8 months old He was coughing and a mild degree of hyperresonance was found over the right lung but in contrast with the usual finding in obstructive emphysema the breath sounds over the right lung were intensified No rales were heard There was no dyspnoea and the apex of the heart was found in its normal position The remainder of the clinical examination was non-contributory No history of foreign body aspiration could be obtained from the family

At fluoroscopy there was mediastinal shift to the left during expiration the mediastinum did not return to the normal median position Lateral films confirmed increased

The autopsy of the first case showed the late morphological changes after cord injury, i e severe segmental atrophy with neurons replaced by glio fibrous tissue adherent to the surrounding tissue The cerebral changes found, indicating anoxic ischemic damage, were either agonal or effects of her respiratory insufficiency

Cesarean section would prevent spinal cord injuries but it is not known how to select cases in breech position at risk Cesarean section is absolutely indicated if hyperextension of the foetal head is demonstrated radiologically after labour has commenced (9) Treatment of already injured children is only supportive No improvement is achieved by laminectomy (9, 12) as shown by the first case but to exclude a spinal cord tumour explorative surgery is sometimes indicated Orthopaedic stabilizing procedures are usually necessary The repeated respiratory and urinary tract infections will require antibiotic therapy

REFERENCES

1 Brakemann O Haltung und Konfiguration des kindlichen Kopfes bei der Beckenendlage *Z Geburtshilfe Perinatol 112* 154 1936

2 Coutelle C Über epidurale Blutungen in den Wirbelkanal bei Neugeborenen und Säuglingen und ihre Beziehung zu anderen perinatalen Blutungen *Z Geburtshilfe Perinatol 156* 19 1961

3 Crothers B Injury of the spinal cord in breech extractions as an important cause of foetal death and paraplegia in childhood *Am J Med Sci 165* 94 1923

4 Crothers B & Putnam M C Obstetrical injuries of the spinal cord *Medicine 6* 41 1927

5 Duncan J M Laboratory note on the tensile strength of the fresh adult foetus *Br Med J II* 763 1874

6 Ford F R Breech delivery in its possible relations to injury of the spinal cord *Arch Neurol 14* 742 1925

7 Hagberg B Olow I & Hagberg G Decreasing incidence of low birth weight diplegia—an achievement of modern neonatal care? *Acta Paediatr Scand 62* 199 1973

8 Hausbrandt F & Meier A Zur Kenntnis der geburtstraumatischen und extrauterin erworbenen Schaden des Zentralnervensystems bei Neugeborenen *Frankfurt Z Path 49* 22 1936

9 Hellstrom B & Sallmander U Prevention of spinal cord injury in hyperextension of the fetal head *JAMA 204* 107 1968

10 Jellinger K & Schwingshackl A Birth injury of the spinal cord *Neuropaediatrie 4* 111 1973

11 Knowlton R W Flying foetus *J Obstet Gynaecol Br Commonw 45* 834 1938

12 Leventhal H R Birth injuries of the spinal cord *J Pediatr 56* 447 1969

13 Melchior J C & Tygstrup I Development of paraplegia after breech presentation *Acta Paediatr Scand 52* 171 1963

14 Muller K M & Lobker U Perinatale kindliche Wirbelsaulenverletzungen *Zentralbl Allg Pathol 114* 209 1971

15 Parrot T Note sur un cas de rupture de la moelle chez un nouveau né par suite de manœuvres pendant l accouchement *L Union med II* 137 1870

16 Pierson R N Spinal and cranial injuries of the baby in breech deliveries A clinical and pathological study of thirty-eight cases *Surg Gynecol Obstet 37* 80? 1923

17 Schulman S T Madden J D Esterly J R & Shanklin D R Transection of spinal cord a rare obstetrical complication of cephalic delivery *Arch Dis Child 46* 291 1971

18 Stern W E & Rand R W Birth injuries to the spinal cord A report of 2 cases and review of the literature *Am J Obst Gynecol 78* 498 1959

19 Tedeschi C G Preliminary considerations and examination of the skull *In* C G Tedeschi (ed) *Neuropathology Methods and Diagnosis* Little Brown and Co Boston 1970 p 43

20 Towbin A Spinal cord and brain stem injury at birth *Arch Pathol 77* 620 1964

21 — Latent spinal cord and brain stem injury in newborn infants *Dev Med Child Neurol 11* 54 1969

22 — Central nervous system damage in the human fetus and newborn infant *Amer J Dis Child 119* 529 1970

23 Yates P O Birth trauma to vertebral arteries *Arch Dis Child 34* 436 1959

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Fig 1 (*a*) PA film at 7 months of age Mediastinal shift to the left with increased radiance of the upper lung field fluoroscopic manifestations of obstructive emphysema No evidence of atelectasis (*b*) Lateral film added one month later emphysema of lung apex still present

air content in the right upper lung without disclosing areas of atelectasis elsewhere (Fig 1*b*)

As the general condition of the infant was good and the pulmonary findings were static he was given 5 days of *therapeutic trial* consisting of increased humidity (tent) and antibiotics (ampicillin and sodium cloxacillin) It was considered that this therapeutic approach might be effective should the symptoms be the consequence of mucus plug When fluoroscopic and roentgenographic examinations were repeated a week later there was no change in the original findings

Bronchography revealed a tracheal bronchus arising 1 5 cm from the carina (Fig 2) connecting with the apical segment which was of increased volume with splaying of the bronchi Nothing in the calibre and course of this bronchus could predispose to impaired aeration The remainder of the bronchial tree was normal the right upper lobe bronchus led only to the posterior and a diminutive anterior segments The main bronchi and oesophagus showed no impressions of anomalous coursing vessels (4)

The clinical symptoms subsided gradually one month later the child was found to be in excellent condition breath sounds over the right lung were still harsher than over the left one and the increased transradiancy of the right lung with the mediastinum displaced toward left were unchanged

DISCUSSION

Embryological considerations

The origin of the respiratory tract is from the foregut, beginning in the fourth week when the embryo is 3 mm long During its early development the foregut divides into a posterior part from which the gastrointestinal tract develops and an anterior one which is the future respiratory tract

The tracheobronchial diverticulum which originates in the pharynx subsequently divides into two primary bronchial branches These primary bronchial branches penetrate the mesenchyme and during their growth further ramification into segmental bronchi bronchioli terminal bronchi and alveoli takes place (7)

According to Bremer (1) the tracheal bronchus is a stage of normal development of the tracheal bronchial tree in the first weeks of life being usually resorbed in humans and persisting as a normal anatomical feature in some mammals (8) Absence of its resorption is responsible for tracheal bronchi diverticuli accessory lung or bronchial cyst (2)

Two anatomical variants have been reported the *supernumerary* variant in which the tracheal bronchus is an additional airway also supplying the right lobe the *apical displaced* variant in which the bronchus to the right apical segment arises from the trachea (Fig 3) The apical displaced tracheal bronchus as in this case is the more frequent form (5) Another classification relates to the distance of the supernumerary or displaced bronchus from the carina the carinal and



Fig 2 (*a*) Apical displaced tracheal bronchus (in oblique projection) of even calibre throughout (*b*) Lateral projection The tracheal bronchus supplying the emphysematous lung apex (cf Fig 1*b*) The diminutive right upper lobe bronchus connects only with the posterior and part of the anterior segments (→)

the tracheal (more than 1 cm from the carina) (6) Very rarely the supernumerary variant of tracheal bronchus may be found together with a supernumerary apical lobe separated from the remainder of the upper lobe by a fissure and different from the azygos lobe The apical displaced variant is usually found to be connected to the right apical segment

Pathogenesis and management

The recurrence of clinical findings suggestive of bronchopneumonia prompted the roentgenographic examination of the chest in this patient There was no detectable left atelectasis while mediastinal shift to the left accentuated on *inspiration* seemed inconsistent with obstructive emphysema on the right This radiological uncertainty and the clinical setting favouring respiratory infection led to the decision to resort to bronchography rather than bronchoscopy



Fig 3 Sketch of bronchial distribution to right upper lobe (*a*) Normal (*b*) Supernumerary bronchus (*S B*) (*c*) Displaced bronchus (*D B*)

Tracheal bronchus is usually an incidental finding with no clinical significance However there is a small number of cases on record of adults with symptoms such as persistent cough expectoration and recurrent infections of the right upper lobe without proved causative relation (6)

To our knowledge isolated tracheal bronchus has not been reported in infancy and childhood Nowhere is it mentioned in the differential diagnosis of mediastinal shift

The present case suggests that an apical displaced bronchus may bring about increased air content of the right upper lung with mediastinal shift to the left The shorter distance and fewer subdivisions from origin to terminal bronchioles in this airway could explain the greater lung volume and asymmetrical ventilation

As a matter of policy it would seem that where foreign body aspiration is doubtful bronchography being less traumatic and more informative should precede bronchoscopy

REFERENCES

1 Bremer J L Accessory bronchi in embryos their occurence and probable fate *Anat Rec* *54* 361 1932
2 — *Congenital anomalies of the viscera Their embryological basis* Harvard University Press Cambridge Massachusetts 1957
3 Caffey J *Pediatric X ray diagnosis* Year Book Medical Publishers Inc Chicago 1972 6th ed p 332
4 Capitanio M A Ramos R & Kirkpatrick J A Pulmonary sling *Am J Roentgenol Radium Ther Nucl Med 112* 28 1971
5 Foster Carter A F broncho-pulmonary abnormalities *Br J Tuberculosis 40* 111 1946
6 Harris J H The clinical significance of the tracheal bronchus *Am J Roentgenol Radium Ther Nucl Med 79* 228 1958
7 Harrison R G *A textbook of human embryology* Blackwell Scientific Publications Oxford 1959 p 103
8 Warkany J *Congenital malformations* Year Book Medical Publishers Inc Chicago 1971 p 600

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CASE REPORT

MALDESCENT OF THE THYMUS IN A HYPOPARATHYROID INFANT WITH PHARYNGEAL POUCH SYNDROME

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ABSTRACT Vesterhus P Eide J Frøland S S Haneberg B and Jacobsen K B (Department of Paediatrics and Department of Pathology the Gade Institute University of Bergen Bergen and Institute of Immunology and Rheumatology Rikshospitalet University Hospital Oslo Norway) Maldescent of the thymus in a hypoparathyroid infant with pharyngeal pouch syndrome Acta Paediatr Scand 64 555 1975.—The clinical and pathological findings in a 6-month-old boy with III–IV pharyngeal pouch syndrome are reported The infant had multiple congenital anomalies including absence of the parathyroid glands maldescent of the thymus aberrant right subclavian artery and dysfunction of the glossopharyngeal nerve Because of persistence of the thymus in the cervical area a thymic shadow was not found on roentgenographic examination of the anterior mediastinum The weight and histology of the thymus were normal as were studies of humoral and cellular immunity

KEY WORDS Hypoparathyroidism thymus pharyngeal pouch syndrome

Primary hypoparathyroidism has been found in hereditary and sporadic forms and at different ages In a familial type often associated with Addison s disease and moniliasis hypoparathyroidism becomes manifest after the first year of life In some male patients diagnosed in the first year of life the disease seems to be sexlinked recessive Another non familial type affecting both sexes with hypoparathyroidism beginning in the first months of life has usually been associated with other congenital anomalies such as thymus dysplasia malformed ears anomalies of facial and cranial bones esophageal atresia cardiovascular cerebral and optic anomalies This constellation has been termed III–IV pharyngeal pouch syndrome (DiGeorge) There has been considerable interest in the immunologic significance of thymic deficiency in these patients since they represent an experiment by Nature (8) However as demonstrated in the present case there are a variety of clinical patterns associated with malformations of the derivatives of the pharyngeal pouches (10)

CASE HISTORY

T C a 16-hour old male infant was admitted to the Children s Hospital in Bergen because of perinatal asphyxia Both parents two sisters and a brother were healthy The mother was 4 years old Pregnancy was normal and ended after 38 weeks in a spontaneous labour The birth weight was 2750 g and the length was 47 cm The patient had malformed ears (Fig 1) micrognathia a minute palatal cleft left sided choanal atresia a large coloboma of the left optic fundus overriding fingers and muscular hypertonia The otolaryngologist reported paresis of the glossopharyngeal nerves Absence of acusticopalpebral reaction indicated deafness The ear drums seemed normal There

REFERENCES

1 Bremer J L Accessory bronchi in embryos their occurence and probable fate *Anat Rec 54* 361 1932
2 — *Congenital anomalies of the viscera Their embryological basis* Harvard University Press Cambridge Massachusetts 1957
3 Caffey J *Pediatric X ray diagnosis* Year Book Medical Publishers Inc Chicago 1972 6th ed p 332
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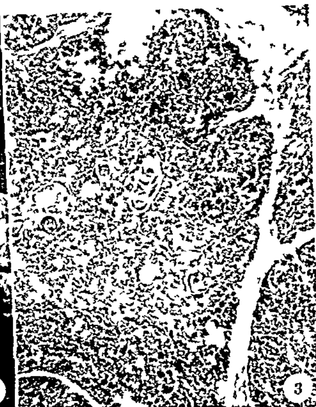


Fig 2 Thoracal organs view from behind Arrows pointing to the two ectopic thymus nodules and the anomalous right subclavian artery

Fig 3 Section of thymus showing normal corticomedullary differentiation and Hassall s bodies (H&E ×70)

and IgA by single radial immunodiffusion at birth 3 weeks and 4 months of age was normal The nitroblue tetrazolium test of the granulocytes was normal

Autopsy

A necropsy was performed ?0 hours after death The following developmental anomalies were found

Absence of the parathyroid glands These were investigated macro- and microscopically in relation to a normal thyroid gland and the tissues of the thorax The tissues of the neck and the thorax were totally embedded in several paraffin blocks of 1×1×0 5 cm From ea h specimen 3 sections were cut at 3 levels and stained with hematoxylin eosin No parathyroid tissue was fou d

Ectopic localiz.ati n of the thymus gland There was no thymic tissue in the anterior mediastinum Thymic tissue was represented by two ectopic nodules One nearly 2 g in weight was found behind the esophagus at the level of the upper part of the thyroid cartilage The other half the size lay to the right of the first (Fig) Histological investigation showed normal corticomedullary differentiation and Hassall s bodies In certain cases the Hassall s bodies showed aggregation (Fig 3)

The right subclavian artery originated just below the left subclavian artery and crossed the midline behind the esophagus (*Dysphagia Lusoria*) (Fig ?) The right vertebral artery originated from the right internal carotid artery immediately after the bifurcation of the common carotid artery Lymph nodes were present in the neck the thorax the root of the mesenteric and in the omentum They were moderately enlarged Histology showed normal differentiation into cortical and paracortical areas with distinct germinal centres Some showed marked reticulum cell hyperplasia The ilal Peyer s plaques and lymphoid tissue in the appendix were normal

The lungs showed increased weight and areas of increased consistency Histology showed pneumonia with a predominance of mononucelar cell infiltration

In the collecting tubules of the kidneys there were mall foci of calcification

The lungs some of the lymph nodes the spleen and the liver showed foci of extramedullary myelopoiesis The bone marrow was normal No other signs of disease or developmental anomalies were found Unfortunately permission to open the cranium was not obtained

DISCUSSION

Congenital hypoparathyroidism beginning in the first year of life is rare Up to 1966 only 20 cases had been reported (10) Eight of 12 autopsy reports on patients with primary



Fig 1 The patient at 3 months of age Note low set and malformed ear and hypoplastic mandible

was moderate respiratory distress and a cyanotic episode with excessive amounts of mucus in the oropharynx Roentgenograms of the heart and lungs were normal but the thymic shadow was not seen There was a retro-esophageal indentation interpreted as secondary to an aberrant right subclavian artery A tracheo-esophageal fistula was not found Intravenous pyelography was normal No bony abnormalities were noted The electro-cardiogram was normal

At admission the serum calcium was 7 8 mg/100 ml Treatment was started with calcium lactate orally but the next day at 30 hours of age the patient had a generalized seizure and the serum calcium was 6 6 mg/100 ml Serum phosphorus was 9 2 mg/100 ml and serum magnesium 1 9 mg/100 ml Other serum electrolytes acid–base balance arterial oxygen saturation and blood glucose were normal Spinal fluid and urine analysis and paper chromatography of the urine for amino acids also showed normal results The hemoglobin was 18 4 mg/100 ml and white cells 14 200/mm³ with a differential count of 84 neutrophils 12 lymphocytes and 4 eosinophils Serum electrophoresis blood urea nitrogen and alkaline phosphatase were normal To keep the serum calcium within normal limits large doses of calcium lactate orally and intravenous calcium injections were necessary The serum phosphorus re mained elevated and urinary 24 hour excretion of calcium and phosphorus were diminished Chromosome analysis revealed a normal karyotype The working diagnosis was a pharyngeal pouch syndrome with multiple anomalies in cluding congenital hypoparathyroidism thymic dysplasia and possibly immunologic deficiency

The infant was strictly isolated Dysphagia was a serious problem He was usually fed by gastric tube He had several cyanotic aspiration episodes and was twice resus citated Candidiasis was not present The serum calcium was normal with oral calcium lactate and vit D daily The electro encephalogram was normal His development was retarded but the general condition gradually improved At 6 months of age he had a severe pneumonia from which he recovered slowly but then he suddenly died during a cyanotic episode probably due to aspiration

Endocrine studies

At 2 weeks of age parathyroid extract (Lilly) 70 USP units was given by intramuscular injection every 12 hours for 3 days The serum calcium rose from 5 8 to 10 4 mg/100 ml and the serum phosphorus fell from 10 0 to 6 9 mg/100 ml The urinary 24 hour excretion of phosphorus increased from 3 6 mg before the test to 81 mg the last 24 hours of the test period After the test the serum calcium and phosphorus returned to abnormal values Following treat ment with oral calcium lactate and 30 000 units of vitamin D_2 (calciferol) daily there was a rise of serum calcium to normal stable values and a decline of serum phosphorus Serum immunoreactive parathyroid hormone (iPTH) was undetectable in the patient at an age of 5 months when serum calcium was 9 5 mg/100 ml phosphorus 5 5 mg/100 ml and magnesium 2 51 mg/100 ml (Laboratory of C D Arnaud Department of Endocrine Research Mayo Clinic Rochester) Parathyroid hormone in a serum sample from a control sent concomitantly was normal Adrenal and thyroid function tests and sweat iontophoresis were nor mal The serum calcium and phosphorus of the parents were normal

Immunological studies

Delayed hypersensitivity to *Candida albicans* antigen was negative on skin testing A positive skin test to dinitro-chlorobenzene was obtained after epicutaneous stimula tion The absolute blood lymphocyte counts ranged from 1 700/mm³ at birth to 4 060/mm³ at 5 months of age Three weeks after birth lymphocyte subpopulations in the pe ripheral blood were evaluated by lymphocyte markers B lymphocytes were identified by their membrane bound Ig detected by immunofluorescence staining (3) The total percentage of Ig positive lymphocytes was 16% which is normal (normal range[1] at birth 5–33% in adults 3–22%) T lymphocytes were identified by receptors for sheep eryth rocytes as detected by rosette formation (4) The patient had 23% rosette forming T lymphocytes in peripheral blood which is within a normal range with the rosette technique used (normal values[1] 5–33%) Also Fc re ceptor bearing like cells in the blood were determined by rosette technique as described elsewhere (5) Twelve per cent of lymphocyte like cells were detected by this test which is within the normal range[1] (5–40%)

Lymphocyte activity *in vitro* was also evaluated 3 weeks after birth DNA synthesis was measured after culture of lymphocytes with phytohemagglutinin (PHA) pokeweed mitogen (PWM) and mitomycin C treated allogeneic lymphocytes in mixed lymphocyte culture (MLC) The culture technique has been described in detail elsewhere (3) The lymphocytes showed normal reactivity with both PHA and PWM There was also a good response of the patient s lymphocytes on stimulation with allogeneic cells in MLC Quantitation of serum concentrations of IgG IgM

[1] Values at Institute of Immunology and Rheumatology Rikshospitalet University Hospital Oslo





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CASE RFPORT

PLASMA ADRENALIN IN A CHILD WITH KETOTIC HYPOGLYCEMIA AND CALCIFICATIONS OF THE SUPRARENAL GLANDS

B BROCK JACOBSEN K W KASTRUP and N JUEL CHRISTENSEN

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ABSTRACT Jacobsen B B Kastrup K W and Christensen N J (Childrens Hospital Fuglebakken Copenhagen and 2nd Clinic of Internal Medicine Kommunehospitalet Aarhus Denmark) Plasma adrenalin in a child with ketotic hypoglycemia and calcifications of the suprarenal glands. Acta Paediatr Scand 64 559 1975.—Urinary excretion of adrenalin has been reported to be reduced during insulin induced hypoglycemia in a significant proportion of children having ketotic hypoglycemia By employing a sensitive double-isotope derivative technique plasma adrenalin and plasma noradrenalin were determined in a boy 6 years 9 months old who had had ketotic hypoglycemia with intermittent hypoglycemic symptoms from the age of 10 months. Bilateral calcifications of the suprarenal glands were present The adrenocortical function was normal The plasma adrenalin response to hypoglycemia were practically absent being only 4% of the value obtained in healthy children The results were related to previous findings of a low plasma adrenalin response in patients with ketotic hypoglycemia without adrenal calcifications and support the assumption that ketotic hypoglycemia is associated with hypoadrenalinemia

KEY WORDS Ketotic hypoglycemia hypoadrenalinemia suprarenal calcifications

The syndrome of idiopathic spontaneously occurring hypoglycemia in infants was first described by McQuarrie (14)

The vast majority of these children present a fairly characteristic disorder called ketotic hypoglycemia (7 15) A significant proportion of these children have a reduced urinary output of epinephrine during insulin induced hypoglycemia first described by Broberger & Zetterstrom (2 3) and later verified by many investigators (4 9 13 18)

Results of several studies indicate that ketotic hypoglycemia and spontaneous hypoglycemia associated with adrenal medullary hyporesponsiveness are one and the same disease (6 13 18)

Employing a precise and sensitive double isotope derivative technique for the determination of adrenalin Christensen (6) has recently shown that plasma adrenalin levels did not rise in a small group of children with ketotic hypoglycemia during insulin induced hypoglycemia

We have recently seen a child with spontaneous hypoglycemia and bilateral calcifications of the suprarenal glands The child had also a severely impaired plasma adrenalin response to hypoglycemia Adrenocortical insufficiency was not present

This case was presented in part at the 13th meeting of European Society for Paediatric Endocrinology in Paris 1974

hypoparathyroidism without Addison s disease collected by Taitz et al (9) probably belonged to the pharyngeal pouch group These patients often also have associated anomalies of the derivatives of the pharyngeal arches Thus the parathyroid glands and the thymus are derived from the III and IV pharyngeal *pouches* whereas the external ears and the mandible are derivatives of the I and II pharyngeal *arches* The dysphagia encountered in our patient could be explained by glossopharyngeal nerve dysfunction or perhaps by the aberrant right subclavian artery (Dysphagia Lusoria) which is the most common anomaly of the aortic arch but rarely causes difficulties in swallowing (1) The glossopharyngeal nerve is the nervous component of the III pharyngeal arch Anomalous right subclavian artery and other vascular anomalies of the derivatives of the III and IV branchial arteries are often found concomitant with abnormalities of the thymus (2)

A very careful post mortem examination of the neck and the thorax demonstrated absence of the parathyroid glands and confirmed the clinical diagnosis of congenital hypoparathyroidism The demonstration of a thymus with normal weight and histology was consistent with the normal results of the immunological studies particularly the tests aiming at evaluation of T cell functions *In vitro* tests of lymphocyte functions and the use of lymphocyte markers to determine the proportions of T and B lymphocytes in blood excluded the presence of a thymus dysplasia with profound and selective T cell deficiency Since immunological reconstitution may now be attempted in profound cell mediated immune deficiency states e g thymus transplantation in DiGeorge syndrome, a precise immunological assessment and functional diagnosis is of more than academic interest in these disorders Furthermore the suspicion of a profound lack of T cell functions should lead to the utmost caution in the use of routine blood transfusions (6)

Any neonatal stressfull illness may result in involution of the thymus In our patient the thymic shadow was not evident in roentgenographic examination of the retrosternal space because of thymic ectopia Similar abnormal positions of the thymus have been reported (7) The possibility of maldescent of an otherwise normal thymus should be taken into consideration when the diagnosis of immunodeficiency is entertained in infants without thymic shadows on repeated roentgenographic examinations

The present report demonstrates that thymic deficiency with immunologic abnormality is not obligatory in III–IV pharyngeal pouch syndrome with an absence of the parathyroid glands The eponyma DiGeorge syndrome therefore does not cover the present and similar patients

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REFERENCES

1 Caffey J *Pediatric X ray diagnosis* Lloyd Luke Ltd London 1973 6th edition p 1473
2 Cameron A H Malformations of the thymus and cardiovascular system *Arch Dis Child 40* 334 1965
3 Froland S S & Natvig J B Surface bound immunoglobulin on lymphocytes from normal and immunodeficient humans *Scand J Immunol 1* 1 197?
4 Froland S S Binding of sheep erythrocytes to human lymphocytes A probable marker of T lymphocytes *Scand J Immunol 1* 269 1972
5 Froland S S Wisloff F & Michaelsen T E Human Lymphocytes with Receptors for IgG A Population of Cells Distinct from T and B Lymphocytes *Int Arch Allergy* (in press)
6 Hong R Gatti R A & Good R A Hazards and potential of blood transfusion in immunologic deficiency *Lancet II* 388 1968
7 Lewis M R Persistence of the thymus in the servical areas *J Pediatr 61* 887 1962
8 Lischner H W DiGeorge syndrome(s) *J Pediatr 81* 1042 1972
9 Taitz L S Zarate Salvador C & Schwartz E Congenital absence of the parathyroid and thymus glands in an infant *Pediatrics 38* 412 1966
10 Warkany J *Congenital malformations Notes & comments* Year Book Medical Publishers Inc Chicago 1971 pp 446 740

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18) although in this disease the hypoglycemic attacks occur more frequently Tietze et al (18) and Sizonenko et al (16) considered it likely that the abnormality was of central nervous origin

It is possible that several mechanisms can impair adrenal medullary secretion and in our patient the abnormality may be related to the bilateral adrenal calcifications While the vast majority of children with ketotic hypoglycemia show hypoadrenalinemia calcifications of the suprarenal glands are usually not present in these children

In a previous study of 4 children with ketotic hypoglycemia and hypoadrenalinemia calcifications were searched for in 3 of these children but none were found (6)

REFERENCES

1 *Black J & Williams D I Natural history of adrenal* haemorrhage in the newborn *Arch Dis Child 48* 183 1973

2 Broberger O Jungner I & Zetterström R Studies in spontaneous hypoglycemia in childhood *J Pediatr 55* 713 1959

3 Broberger O & Zetterstrom R Hypoglycemia with inability to increase the epinephrine secretion in insulin induced hypoglycemia A further report *J Pediatr* 59 ?15 1961

4 Brunjes S Hodgeman J Nowack J & Johns V J Adrenal medullary function in idiopathic spontaneous hypoglycemia of infancy and childhood *Am J Med 34* 168 1963

5 Christensen N J Plasma noradrenaline and adrenaline in patients with thyreotoxicosis and myxoedema *Clin Sci 45* 163 1973

6 — *Hypoadrenalinemia during insulin hypoglycemia in* children with ketotic hypoglycemia *J Clin Endocrinol Metab 38* 109 1974

7 *Colle E & Ulstrom R A* Ketotic hypoglycemia *J Pediatr 64* 632 1964

8 Gardner L I Newer knowledge of adrenocortical disturbances *Pediatr Clin North Am 4* 895 1957

9 Goodall M Cragan M & Sidbury J Decreased epinephrine excretion in idiopathic hypoglycemia *Am J Dis Child 123* 569 1972

10 Hanssen K F Radioimmunoassay of growth hormone *in human plasma. Acta Endocrinol (Kbh) 71* 649 197?

11 Jarvis J & Seaman W B Idiopathic adrenal calcification in infants and children *Am J Roentgenol 82* 510 1959

12 Jørgensen K R Evaluation of the double antibody radioimmunoassay of insulin and the determination of insulin in plasma and urine in normal subject *Acta Endocrinol (Kbh) 68* 371 1969

13 Koffler H Schubert W K & Hug G Sporadic hypoglycemia Abnormal epinephrine response to the *ketogenic diet or to insulin J Pediatr 78* 448 *1971*

14 McQuarrie I Ulstrom R A & Ziegler M R Random notes concerning the ethiological mechanisms and treatment of spontaneous hypoglycemia *Acta Paediatr Scand 43* 481 Suppl *100* 1954

15 Pagliara A S Karl I E Haymond M & Kipnis D A Hypoglycemia in infancy and childhood Part II *J Pediatr 82* 558 1973

16 Sizonenko P G Pauneir L Vallotton M B Cuendet G S Zahnd G & Marlis E B Response to 2 Deoxy D-glucose and to glucagon in Ketotic *Hypoglycemia of childhood Evidence for* epinephrine deficiency and altered alanine availability *Pediatr Res 7* 983 1973

17 *Snelling C E & Erb I H* Hemorrhage and subsequent calcification of the suprarenal *J Pediatr 6* 2? 1935

18 Tietze H U Zurbrugg R P Zuppinger K A Joss E E & Kaser H Occurrence of impaired cortisol regulation in children with hypoglycemia associated with adrenal medullary hyporesponsiveness *J Clin Endocrinol Metab 34* 948 1972

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Table 1 *Plasma catecholamines before and during insulin induced hypoglycemia in the patient compared to normal children*

	Plasma adrenalin (ng/ml)		Plasma noradrenalin (ng/ml)	
	Before	During	Before	During
Patient	0 01	0 04	0 25	0 63
Normal children mean ($n=3$)	0 22	1 33	0 23	0 25

LABORATORY METHODS

Plasma glucose was determined with a glucose oxidase method Plasma insulin and growth hormone were assayed by radioimmunological technique (10 12) Plasma adrenalin and plasma noradrenalin were determined by a double isotope derivative technique described previously (5) Cortisol secretion rate was determined by K Petersen M D Frederiksberg Hospital Copenhagen

CASE REPORT

J T a boy was born at term birthweight 2300 g birth length 45 cm The pregnancy was uneventful He was born in breech presentation and was severely asphyxiated Intensive resuscitation was necessary He recovered slowly but his development was retarded

At 10 months of age he was admitted for the first time with hypoglycemic seizures and since then he has been hospitalized several times for the same reason Usually the attacks occurred in the morning before breakfast Ketonuria has been found during hypoglycemic attacks and during fasting At the age of 5 years radiological examination revealed numerous calcifications in both suprarenal glands

At the age of 6 years 9 months he was readmitted for further investigations The body weight was 20 kg and the height was 20 cm below average for age The bone age was 2 years retarded The psychological tests revealed evidence of cerebral dysfunction The electro encephalogram was normal Neurological examination was repeatedly reported to be normal

Diagnosis of hypoglycemia

After prolonged fasting hypoglycemic symptoms and ketonuria occurred after 17 hours Plasma glucose was 45 mg/100 ml The symptoms disappeared soon after intake of carbohydrates

Adreno cortical function

The cortisol secretion rate was normal (8 4 mg/24 hours) and increased to 93 mg/24 hours after 2 days of ACTH stimulation (Synacthen® 0 5 mg per day)

Further investigations

Plasma insulin concentration was normal in the fasting state and during hypoglycemic attack Leucine test as well as galactose and fructose tests gave normal results A normal response of blood glucose to i m glucagon (30 μg/kg body weight) was obtained before and after 1? hours fasting Plasma growth hormone response to the insulin induced hypoglycemia was normal peak value 36 ng/ml Plasma thyroxine 91 nmol/l and TSH 2 6 mU/l both were normal

Plasma catecholamine study

After 12 hours of fasting crystalline insulin (Leo) 0 05 IU/per kg body weight was given intravenously After ?5 minutes the plasma glucose decreased to 25 mg/100 ml and the test had to be interrupted due to severe hypoglycemic symptoms The results of the determinations of catecholamines in plasma are given in Table 1

The plasma adrenalin response is markedly pathological The expected value in a normal child with a blood glucose concentration of 25 mg/100 ml is approximately 1 10 ng/ml (6)

DISCUSSION

Our patient demonstrated a clinical disorder with many of the features which characterize ketotic hypoglycemia The child showed bilateral calcifications of the suprarenal glands but adrenal cortical insufficiency could be excluded In addition the plasma adrenalin response to hypoglycemia was practically absent being only 4% of the value obtained in healthy children

Calcification of the adrenals in children is a very unusual finding at autopsy or at X ray examination (11 17) Calcifications can be caused by a number of diseases Often the cause has been believed to be a consequence of suprarenal hemorrhages due to fetal distress and traumatic delivery (1 11 17) Many of the children are asymptomatic although the occurrence of hypoglycemia has been described (1 8)

In has been suggested that hypoglycemia associated with adrenal medullary hyporesponsiveness and ketotic hypoglycemia are one and the same disease (6 13 16 18) The cause of the hypoadrenalinemia remains obscure however It is unlikely that the abnormally reduced adrenalin secretion is a consequence of recurrent hypoglycemic attacks *per se* one of the reasons being that a similar abnormality is not present in children with the leucine sensitivity type of hypoglycemia (3

M Iivanainen *A study on the origins of mental retardation* Clinics in Developmental Medicine No 51 Spastics International Publications William Heinemann Medical Books Ltd London 1974 172 pp illus £4 40

This book describes the neurological and neuroradiological findings in 338 patients with mental retardation selected from an institution in Finland with a total of 1 000 patients one to forty nine years old with severe mental retardation The group of 338 was selected according to the existence of focal neurological signs and epilepsia and in this group a very high percentage had positive findings on skull X ray angiographic and pneumoencephalographic studies

The author tries to compare his own material with other series on mental retardates published but points to the great difficulties because most materials are obviously selected in different ways His own material is well described and the angiographic and pneumatoencephalographic pictures are clarified by diagrams The patients are grouped according to two different types of classification of mental retardation and the roentgenological findings in these groups are compared which sometimes makes the results and discussions difficult to understand The author points to the value of diagnosing the anatomical defects in mental retardation and the possibilities this yields towards finding out when the lesions arose The book is of apparent value to those interested in the pathology of mental retardation

Ingrid Bjerre

Margaret I Griffiths (ed) *The young retarded child Medical aspects of care* Churchill Livingstone Edinburgh and London 1973 227 pp illus £3 00

This book is a short but rather comprehensive study of mental retardation written mostly for those who now and then have such patients Several chapters deal with the special organization for children with mental retardation in the United Kingdom and does not have so much interest in other countries

More interesting are the discussions of etiology social factors and early diagnosis—these are vast fields—here shortly but well described Because there are thirteen different authors the quality between the different chapters are changing and some of the information overlapping In my opinion too much space is given to antiepileptic treatment which is not specific for children with mental retardation and of which better information is given in most textbooks The same holds true for treatment of perinatal complications Although this is very important as a background for mental retardation the actual handling of the complications is not of much interest here and cannot easily be described on a few pages

On the whole however the book is well worth reading It is composed by Margaret Griffiths who has herself written two interesting reviews on the background of mental retardation and the early detection Another advantage with this book is that much space is left for the discussion dealing with prevention of mental retardation The book also has a comprehensive reference list for those who want to read more about the subject

Ingrid Bjerre

BOOK REVIEWS

Richard L Wesenberg *The newborn chest* Harper & Row Publishers Hagerstown (Md) New York Evanston San Francisco London 1973 296 pp illus US$20 00

The inimitable adventure of being born adds many specific threats to the numerous hazards of intra uterine development Directly or indirectly most of these affect the respiratory and circulatory systems which are therefore central fields of interest to the neonatologist A variety of abnormalities appearing in the newborn require radiologic examination chest X ray is often vitally important not only in the diagnosis of these conditions but also during treatment

The Newborn Chest is a monograph on such abnormalities and covers all of the common as well as many unusual neonatal disorders of the lung and heart It is written for both clinicians and radiologists Pathophysiology and clinical findings are described the radiologic findings and differential diagnosis are discussed and exemplified by case reports and a brief account is given of the treatment and prognosis Angiography and other radiologic special procedures are not included

The author has mastered the difficult task of systematising and synthesising the knowledge accumulated in various branches of neonatology References are generously supplied and are up to date The text is lucid and didactically arranged to facilitate a rational approach to the interpretation of neonatal chest radiographs The reproduction of the radiographs is not of the same high quality but is usually sufficient to demonstrate the essential points

The book deserves to be widely read It is recommended particularly to radiologists seeking a modern guide in the diagnosis of neonatal respiratory diseases

Georg Theander

Wallace W McCrory *Developmental nephrology* Harvard University Press Cambridge Mass 1973 $12 –

This book describes the anatomical and physiological development of the kidney during embryonic life immediate postnatal period and childhood until adolescence The author always attempts to relate the physiological development to the anatomical changes which is the logical but often foreseen approach to renal physiology The section on the anatomical development of the kidney during embryonic life is the best part of the book and presents a comprehensive and reasonably up to date survey of the field The illustrations are excellent The fact that the book was published in 1973 and only a few of the references are from a later date than 1969 is a definite disadvantage to the section on renal physiology Thus the concept of glomerular tubular imbalance based on the previous incorrect finding of a low Tm glucose in infancy has now been abandoned due to more reliable techniques for determination of Tm The value of this section lies in the synthesis of the data presented Thus for instance the normal values for the glomerular filtration rate from 0 to 20 years are given The author also discusses the correct references for the various renal functional parameters The book closes with a section on compensatory renal growth Since no evidence is presented for identical stimulus (or at least identical development) as compared with normal growth the section seems to be a little unnecessary in this connection

Anita Aperia

M E Avery & B D Fletcher *The lung and its disorders in the newborn infant* 3rd ed Vol I W B Saunders Company Ltd Philadelphia London and Toronto 1974 361 pp illus £5 35

When Mary Ellen Avery in 1964 introduced her The lung and its disorders in the newborn infant it rapidly became a standard book of reference for neonatologists Now the author has considered the time due for the third edition of her classic When first presented the book filled an important gap since the subject was covered only fragmentarily in reviews and monographs In the past decade however a considerable body of literature has appeared—in reviews monographs and textbooks—on the newborn infant in general and the newborn lung and its disorders in particular With this background it may be pertinent to ask whether there is a true need for another edition of Dr Avery s book The reply is Yes This book is still the most comprehensive presentation of relevant data developmental morphology physiology chemistry and pathology together with thorough clinical descriptions of the diseased states This edition has been prompted by the rapid development both of our background knowledge of e g surfactant production by the developing lung and of practical advances of therapy including continuous distending airway pressure and respiratory therapy together with a more detailed insight into long term prognosis after surviving life threatening neonatal pulmonary disease

The book is informative and detailed without being dull It is authoritative without being dogmatic

The radiologist of the Montreal Children s Hospital Dr Barry D Fletcher has contributed as co author The standard of the X ray pictures is high and the extended chapter on roentgen evaluation of the chest adds to the usefulness of this monograph

Ingemar Kjellmer

M Iivanainen *A study on the origins of mental retardation* Clinics in Developmental Medicine No 51 Spastics International Publications William Heinemann Medical Books Ltd London 1974 172 pp illus £4 40

This book describes the neurological and neuroradiological findings in 338 patients with mental retardation selected from an institution in Finland with a total of 1 000 patients one to forty nine years old with severe mental retardation The group of 338 was selected according to the existence of focal neurological signs and epilepsia and in this group a very high percentage had positive findings on skull X ray angiographic and pneumoencephalographic studies

The author tries to compare his own material with other series on mental retardates published but points to the great difficulties because most materials are obviously selected in different ways His own material is well described and the angiographic and pneumatoencephalographic pictures are clarified by diagrams The patients are grouped according to two different types of classification of mental retardation and the roentgenological findings in these groups are compared which sometimes makes the resu ts and discussions difficult to understand The author points to the value of diagnosing the anatomical defects in mental retardation and the possibilities this yields towards finding out when the lesions arose The book is of apparent value to those interested in the pathology of mental retardation

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Ingrid Bjerre

ANNOUNCEMENTS

Austrian–Swiss–German Society of Paediatric Surgery

The joint meeting of the Austrian–Swiss–German Society of Paediatric Surgery will be held October 4–6 1976 in Innsbruck Austria

Topics (1) Traumatology in infancy and childhood (2) Surgery of lungs and respiratory tract in infancy and childhood (3) Surgery of the colon in infancy and childhood including anal surgery

Half of the time is intended for discussion Those who intend to present a paper should send the abstract not later than April 30 1976 to Prof Dr H Singer Munchen Schwabing (Germany) Prof Dr N Genton Lausanne (Switzerland) Prof Dr H Sauer Graz (Austria) For further information contact Prof Dr H Sauer A-8010 Graz Heinrichstr 31 Austria

International Organisation for the Study of Human Development

The third meeting of the International Organization for the Study of Human Development will be held in Madrid Spain September 22–24 1975 The program will include the history of food production ethical and philosophical considerations of food the associated geographical economical and sociological implications food and health the availability of food various aspects of teaching and research relating food to the human developmental process

Non members are cordially invited Registration fee $50 00 by June 30 1975 please

A limited number of free papers will be selected—250 word abstract by June 1

Address all correspondence to the Executive Secretary D N Walcher M D Human Development Building University Park Pennsylvania 16802 USA

Acta Pædiatr Scand 64 565–573 1975

HEIGHT AND WEIGHT OF SCHOOL CHILDREN AND ADOLESCENT GIRLS AND BOYS IN OSLO 1970

GRO H BRUNDTLAND KNUT LIESTÖL[1] and LARS WALLÖE

From the Oslo City Health Department Section for School Health and the Departments of Physics and Physiology University of Oslo Oslo Norway

ABSTRACT Brundtland G H Liestol K and Walloe L (Oslo City Health Department and Departments of Physics and Physiology University of Oslo) Height and weight of school children and adolescent girls and boys in Oslo 1970 Acta Paediatr Scand 64 565 1975 —Height and weight measurements of the school children of Oslo in 1970 (aged 7 to 19 years) are reported Weights show considerably skewed distributions with long tails towards higher weights Weight percentiles are calculated by interpolation in the empirical distributions Percentiles and tables for both sexes showing height for age weight for height and weight for age are presented A comparison with existing Norwegian data from Sundal 1956 Bergen shows that the application of statistics based on normal distribution for weight has introduced considerable error in these percentiles Oslo children in 1970 are taller by 5–6 cm at age eighteen than USA (Iowa) standards and 4–5 cm taller compared to Tanner s English percentiles Oslo children are also taller than Swedish children and have reached a stature higher than found in any other comparable study

KEY WORDS Growth height weight percentile charts

Anthropological studies have a long tradition in Norway Investigations on school children initiated by Carl Schiotz have been performed regularly within the municipal School Health Services of Oslo for the last fifty years The earlier studies were partly published in English (5) but in the last three decades no published reports are available not even in Norwegian except for brief mention in Udjus study from 1964 on military conscripts (11)

The latest Oslo investigation was carried out in March 1970 and included data on the menarche as well as measurements of height and weight

Tables or diagrams of cross sectional height and weight distributions in children have some use in practical pediatric work The diagrams available and being used in Norway today are based on Sundal s measurements of children in Bergen in the early and mid fifties (9) They include ages only up to 15 years Because of the time elapsed since his study and because of a somewhat unfortunate use of statistics in it it was thought worthwhile to publish diagrams of the height and weight distributions obtained in the present investigation Some of these diagrams will also be published elsewhere for practical pediatric work

MATERIAL AND METHODS

The study was planned to include the total primary and high school population of Oslo Later due to practical difficulties connected with the heavier work load for public health nurses in the peripheral areas of the city it became necessary to limit the field studies in these parts

[1] K L and L W did not enter the investigation till after the collection of the primary data

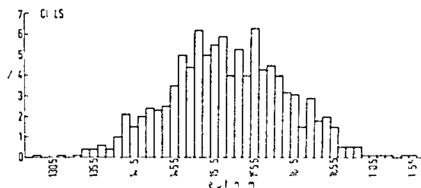


Fig 1 Distribution of heights for 12 year old girls (age 11 years 6 months – 12 years 5 months)

Children from all schools in the peripheral zone entered the study but a selection of classes was performed in cluding in each school approximately one third of the school population at each class level A total of 24 700 children thus entered the study

Up to and including age 16 the data represent the total age group of school attenders Schooling is compulsory for 9 years from age seven to sixteen For ages above sixteen selecting factors are thus involved only high school students (45% of primary schools leavers) being included

The children were measured undressed (except for minimum underwear) by the public health nurse during school hours (8 a m – 2 p m) Weighing was performed on platform scales and the result recorded to the nearest 10 grams Height was measured against the wall on a fixed measuring stick with a perpendicular metal bar The child was asked to straighten the back shoulders touching the wall and to look straight forward Height was recorded to the nearest mm Data were recorded based on a single measurement

For each pupil weight height and age at the time of investigation were punched on a data card together with code numbers indicating sex school and form Calcula tions were carried out on the CDC 3300 computer at The University of Oslo For some of the calculations the height weight and age measures were grouped in suitable groups The values used in the text in the figures and in the tables all refer to group centers The computer was programmed to plot histograms and to calculate percentiles (fractiles) by interpolation in the empirical histograms Means and standard deviations of the dis tributions and percentiles corresponding to the best fitted normal distributions were also calculated Figs 1 2 and 3 show three typical histograms Standard percentile charts were drawn on the basis of percentiles calculated from each distribution (Figs 4 5 6 7 8 and 9) From a methodological point of view the following points are of importance The age–height charts were fitted to percentiles calculated from normal distributions while the height–weight and age–weight charts were fitted to the empirical percentiles directly The reason for this differ ence is that the empirical distributions of heights within each age group are symmetrical and approximately nor mal while the distributions of weights within both age groups and height groups are grossly skewed with long tails towards higher weights These distributions could therefore not be adequately described as normal One of the charts (Fig 8) shows the data points in addition to the percentile lines The figure illustrates that some smooth ing of the data was performed during construction of the charts Smoothing was done visually Fig 8 also shows that the statistical scatter of the data points is greater in the upper and lower percentile points as could be ex pected due to the smaller number of observations The scatter of the data points in the other five charts is less than the scatter shown in Fig 8

RESULTS

Within each age group the distributions of heights are symmetrical and bellshaped One example is shown in Fig 1 The distributions are similar for the other age groups and for

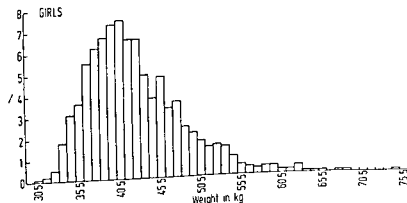


Fig 2 Distribution of weights for girls with height 150 0–154 9 cm

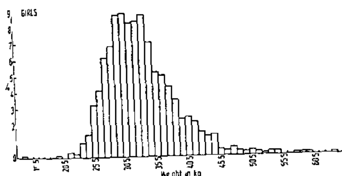


Fig 3 Distribution of weights for 10 year old girls (age 9 years 6 months – 10 years 5 months)

both sexes These empirical distributions could be approximated by normal distributions Within each height group the weights show a skew distribution with a long tail towards higher weights Fig 2 shows a typical example This finding was equally pronounced in all height groups and was equally pronounced for girls and for boys However the distributions for girls are wider have a greater standard deviation in all height groups up to 167 5 cm The differences between the standard deviations amounts to approximately 20% The weights show similar distributions within each age group (Fig 3) The sex differences are also here of magnitude 20% but apply only to ages below 12 years

Table 1 *Height for age Percentiles S D and S E calculated from best fitted normal distributions*

Age	Number of pupils	Mean height (cm)	S D (cm)	? S E (cm)	Percentiles (cm) 0 5	2 5	10	25	50	75	90	97 5	99 5
A *Boys*													
7	235	125 8	4 9	0 64	113 2	116 2	119 5	122 5	125 8	129 1	132 1	135 5	138 4
8	108?	129 7	5 5	0 33	115 5	119 0	122 7	1 6 0	129 7	133 4	136 7	140 4	143 9
9	11?9	135 0	5 9	0 35	119 8	123 6	127 5	131 1	135 0	139 0	142 6	146 5	150 2
10	1068	139 9	6 0	0 37	124 4	128 2	132 3	135 9	139 9	144 0	147 6	151 7	155 4
11	1121	144 7	6 3	0 38	1?8 5	132 4	136 7	140 5	144 7	149 0	152 8	157 0	160 9
12	1073	150 4	7 4	0 45	131 3	135 9	140 9	145 4	150 4	155 4	159 9	165 0	169 5
13	1058	156 4	8 2	0 50	135 3	140 3	145 8	150 9	156 4	162 0	167 0	172 5	177 5
14	11?4	163 7	8 7	0 52	141 3	146 7	152 6	157 8	163 7	169 6	174 8	180 7	186 1
15	1176	170 3	8 2	0 48	149 2	154 4	160 0	164 9	170 3	175 8	180 7	186 2	191 4
16	1130	175 3	7 1	0 42	157 0	161 3	166 2	170 5	175 3	180 1	184 4	189 2	193 6
17	1017	178 5	6 5	0 41	161 8	165 8	170 2	174 2	178 5	182 9	186 8	191 2	195 2
18	722	180 3	6 1	0 45	164 4	168 4	172 5	176 2	180 3	184 4	188 1	19? 2	196 0
19	515	181 0	6 5	0 57	164 3	168 3	172 7	176 6	181 0	185 4	189 3	193 7	197 7
?0	09	181 0	6 5	0 90	164 3	168 3	172 7	176 6	181 0	185 4	189 3	193 7	197 7
B *Girls*													
7	80	125 3	4 9	0 59	112 7	115 7	119 0	122 0	125 3	1?8 6	131 6	135 0	138 0
8	1003	1?8 9	5 5	0 35	114 7	118 0	121 8	1?5 1	1?8 9	132 6	136 0	139 7	143 1
9	10?1	133 8	6 1	0 38	118 1	121 8	126 0	129 7	133 8	137 9	141 7	145 8	149 5
10	1050	139 6	6 2	0 38	123 6	127 4	131 6	135 4	139 6	143 8	147 6	151 8	155 6
11	1116	145 1	7 3	0 46	1?5 3	130 8	135 8	140 2	145 1	150 0	154 4	159 3	164 9
12	10?6	152 0	7 2	0 44	133 5	137 9	142 8	147 2	152 0	156 9	161 3	166 1	170 5
13	1160	158 4	7 0	0 41	140 4	144 7	149 5	153 7	158 4	163 1	167 4	172 1	176 4
14	1099	162 5	6 3	0 38	146 3	150 2	151 5	158 3	162 5	166 8	170 6	174 8	178 7
15	1215	164 7	5 9	0 34	149 5	153 2	157 ?	160 8	164 7	168 7	172 2	176 2	179 9
16	1165	166 4	6 0	0 35	150 9	154 6	158 7	162 4	166 4	170 5	174 1	178 2	181 9
17	1067	166 9	5 7	0 35	15? 2	155 7	159 6	163 0	166 9	170 8	174 3	178 2	181 6
18	770	167 3	5 5	0 40	153 1	156 5	160 2	163 6	167 3	171 0	174 3	178 1	181 5

Table 2 *Weight for height Percentiles are calculated by interpolation in the empirical distributions*

Height (cm)	Number of pupils	Mean weight (kg)	S D (kg)	Percentiles (kg) 2 5	10	25	50	75	90	97 5
A *Boys*										
122 5	309	23 8	2 0	20 3	21 3	22 4	23 7	24 9	26 3	28 4
127 5	632	25 9	2 2	22 1	23 3	24 4	25 7	27 0	28 5	31 1
132 5	962	28 6	2 8	24 2	25 4	26 7	28 3	30 2	31 9	35 [illegible]
137 5	1095	31 3	3 2	26 1	27 6	29 2	30 8	33 0	35 3	38 8
142 5	1066	34 6	3 8	28 7	30 4	32 2	34 1	36 6	39 1	43 8
147 5	106[illegible]	38 0	4 4	31 3	33 3	35 0	37 4	39 9	43 3	47 7
152 5	889	41 9	5 1	34 1	36 2	38 3	41 0	44 4	48 2	55 6
157 5	778	46 0	5 9	37 2	39 5	42 0	45 1	48 7	53 8	60 7
162 5	739	50 6	6 6	40 8	43 5	46 3	49 7	53 4	58 3	66 5
167 5	874	55 1	6 7	44 6	47 5	50 4	54 3	58 7	63 7	70 0
172 5	1150	60 3	6 8	49 4	52 5	55 4	59 4	64 4	69 2	76 4
177 5	1262	64 7	6 9	53 2	56 8	60 1	64 0	68 4	72 8	8[illegible] 8
182 5	999	68 8	6 9	56 7	60 6	64 2	68 4	72 6	77 [illegible]	85 [illegible]
187 5	433	73 0	7 8	59 9	64 4	68 0	72 2	76 9	82 6	92 2
192 5	123	76 6	8 2	62 0	65 6	71 2	76 2	82 0	86 5	94 4
B *Girls*										
122 5	360	23 7	2 2	19 9	21 1	22 2	23 5	25 0	27 6	28 5
127 5	716	26 1	2 9	21 6	23 0	24 2	25 6	27 4	29 8	33 6
132 5	862	28 5	3 3	23 5	25 0	26 3	28 0	30 2	32 7	36 0
137 5	915	31 8	3 7	26 2	27 5	29 2	31 2	33 6	36 6	41 1
142 5	924	34 8	4 6	28 0	29 8	31 8	33 9	37 1	40 7	46 4
147 5	844	38 4	5 2	30 9	32 8	34 9	37 4	41 3	45 3	51 5
152 5	909	42 5	6 1	33 8	35 9	38 2	41 4	45 7	50 7	57 7
157 5	1354	48 3	6 8	37 2	40 4	43 6	47 6	52 0	57 0	65 0
162 5	2034	52 8	7 1	41 5	45 0	48 0	51 8	56 8	61 6	68 9
167 5	1996	56 6	6 7	46 1	49 1	52 0	55 7	60 2	64 9	72 0
172 5	1101	60 2	6 7	48 8	52 4	55 7	59 7	63 9	68 9	75 9
177 5	290	63 9	8 0	51 7	55 8	59 2	63 3	67 8	74 6	80 9

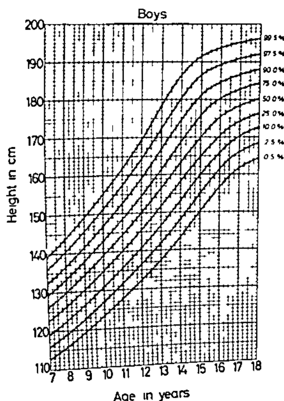


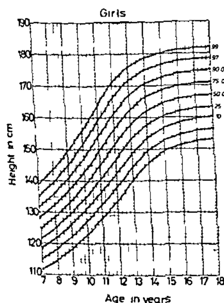
Fig 4 Percentile chart showing height for age boys Data points calculated from best fitted normal distributions and smoothed visually

Conventional percentile charts relating height to age weight to height and weight to age are shown for both sexes in Figs 4 to 9 and in Tables 1 2 and 3 Differences in stature between the sexes (Figs 4 and 5) are minor below the age of ten after which the girls percentiles are ahead of the boys for a period varying from 3 years for the highest percentile up to 4½ years for the lowest

Reflecting the pubertal growth spurt as can also be seen in cross sectional charts the slopes of the percentile lines for height upon age are seen gradually to increase for boys for the highest percentile from age 9 and successively at later ages down to the lowest

Table 3 *Weight for age Percentiles are calculated by interpolation in the empirical distributions*

Age	Number of pupils	Mean weight (kg)	S D (kg)	Percentiles (kg) 2 5	10	25	50	75	90	97 5
A *Boys*										
7	?35	?5 4	3 3	0 3	1 8	?3 4	24 9	26 7	29 3	33 5
8	1082	?7 0	3 8	?1 0	22 7	24 5	26 6	?9 2	31 8	35 4
9	11?9	30 2	4 6	23 1	25 2	27 1	9 6	3? 5	36 0	40 6
10	1068	33 1	5 1	25 1	?7 4	?9 6	3? 4	36 0	40 0	45 1
11	11 0	36 2	5 7	27 2	30 0	32 3	35 6	39 0	43 0	50 0
12	1073	40 7	7 6	?9 5	32 7	35 5	39 5	44 3	50 4	59 0
13	1058	45 2	8 3	32 5	35 6	39 1	44 2	50 3	56 4	65 4
14	11 4	51 3	9 3	36 0	40 0	44 4	50 7	56 9	63 3	71 2
15	1179	57 ?	9 7	40 7	46 3	50 4	56 4	62 8	69 6	79 1
16	1133	6? 5	9 4	45 0	51 4	56 3	61 8	67 9	74 1	83 7
17	1018	65 5	8 0	51 ?	55 4	60 0	65 3	70 5	75 6	8? 7
18	7 4	68 8	8 2	54 5	59 2	63 4	68 2	73 0	79 5	88 2
19	515	70 1	8 3	56 7	60 ?	64 5	69 0	74 6	81 5	88 0
B *G rls*										
7	?80	25 3	3 8	19 5	21 1	?? 7	?4 7	?7 0	30 6	34 7
8	1003	6 8	4 4	0 ?	?2 0	23 9	?6 1	?9 0	32 2	37 3
9	10 1	9 6	5 0	2 2	?4 1	25 9	?8 8	32 4	35 9	42 6
10	1049	33 2	5 8	?4 7	?7 0	29 3	3? 4	36 1	40 5	46 5
11	1117	37 1	7 1	6 5	9 4	32 3	36 0	41 0	46 2	52 6
12	1056	41 4	7 3	?9 7	32 6	36 2	40 6	45 5	?0 8	58 0
13	1160	47 ?	8 4	33 0	36 9	41 3	46 8	?? 0	?8 2	67 0
14	1097	51 8	8 4	37 3	41 9	46 2	50 9	?6 5	62 3	0 8
5	1?15	54 6	8 0	41 2	45 3	49 4	53 9	?9 0	64 3	73 4
16	1164	56 7	7 6	43 6	47 8	51 3	56 0	61 0	66 4	74 7
17	1067	57 ?	7 2	45 3	48 5	52 3	56 4	61 4	66 9	74 6
18	768	57 8	7 1	45 8	49 3	52 8	57 7	62 0	66 9	73 2



ig 5 Percentile chart showing height for age girls Data points calculated from best fitted normal distributions and smoothed visually

percentile where it does not start to increase before age 12 Correspondingly there is a systematic trend of earlier points of deflection for the higher percentiles from age 14 down to age 15 5 for the lowest percentile In girls the points of deflection are found at 11 5 years for the highest percentile and at 13 5 years for the lowest while the start points for the increase in the slopes are more difficult to trace in the girls percentiles since data for younger ages not are available

The figures further indicate that girls level off their growth in height more sharply and at a considerably earlier age than boys the tallest girls already having reached adult stature at the age of 16 Many boys are still growing at the age of 18 and seem to reach their adult height at least 2 years later than the girls

Figs 6 and 7 shows weight percentile curves as a function of height Both charts illustrate the skewness in the weight distribu

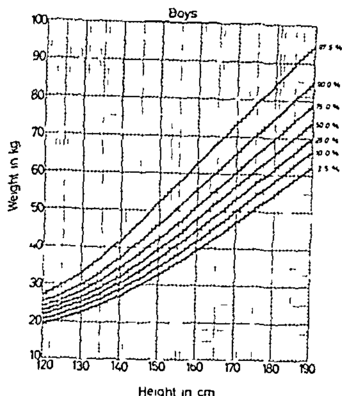


Fig 6 Percentile chart showing weight for height boys Data points calculated by interpolation in the empirical distributions and smoothed visually

tion which was shown in Fig 2 There are obvious differences in shape between the two charts also reflecting the greater weight variability in girls for all heights up to 1 65–1 70 The levels of the 75th and 90th percentiles at *height 1 60 cm are 1 kg and 3 kg respectively* higher for girls than for boys For the tallest girls, the weight percentiles are more similar to the boys

Figs 8 and 9 show weight as a function of age

DISCUSSION

The percentile charts presented apply to the whole age group attending regular schools but do not include children in special schools or in medical institutions For the lowest age group seven years the sample is undoubtedly biased, since it includes the selected group of early school attenders To avoid this bias in practical paediatric work, the height for age diagrams have been adjusted by downward continuation from 8 years The actually measured values are found in the tables For ages seventeen and eighteen selective factors are also involved the data being based only on high school attenders In order to evaluate the magnitude of this effect, comparison with measurements on military conscripts from Oslo has been made The conscripts in 1971 with a mean age of 18 years 9 months had a mean stature of 180 2 compared with 180 3 for our 18 year age group (7) The 19 year-old boys in our study were 181 0 cm The maximum bias introduced would thus amount to less than 3/4 cm

There are considerable differences between *our weight for height percentiles and those of* Sundal (9) The 97 5 percentile for girls with stature 162 5 cm is in Sundal s diagrams, 64 5 kg in ours 68 9 kg the corresponding 2 5 percentile is 38 7 kg versus 41 5 kg The medium are nearly identical 51 6 kg and *51 8 kg* The corresponding mean values however *differ considerably, 51 6 kg versus 52 8 kg* The mean values generally differ by 0 3–1 4 kg Standard deviations in the Oslo data are

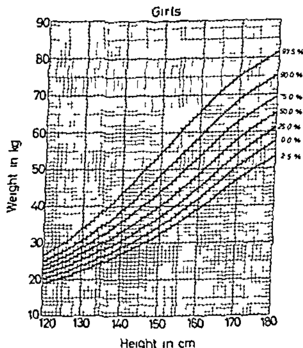


Fig 7 Percentile chart showing weight for height girl Data points calculated by interpolation in the empiric distributions and smoothed visually

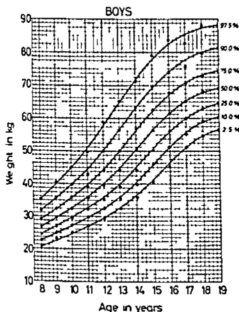


Fig 8 Percentile chart showing weight for age boys Filled circles data points calculated by interpolation in the empirical distributions Lines percentiles smoothed visually

10–20% larger than those of Sundal as they can be determined from the published percentiles

Sundal s tables and diagrams for the distribution of weight for height were unfortunately based on the assumption of normal distributions of weight data To make possible a more direct comparison between the Bergen and Oslo findings and to evaluate the differences in methodology two methods of analysis have been applied In Fig 10 the Oslo data have been treated according to the statistical methods used by Sundal The medium is defined as identical with the mean and the percentiles determined on the basis of normal deviates The use of this method is seen to result in a considerable bias with an underestimate of both the lowest and highest percentiles and an overestimate of the median value The empirical 2 5 and 97 5 percentiles are thus shifted downwards by 1 to 3½ kg the median upwards by ½ to 1 kg

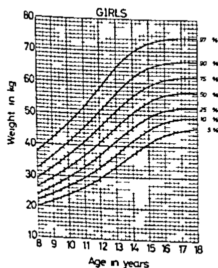


Fig 9 Percentile chart showing weight for age girls Data points calculated by interpolation in the empirical distribu

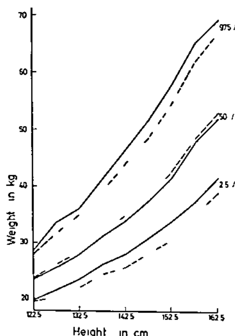


Fig 10 Weight for height percentiles girls Oslo 1970 — percentiles calculated by interpolation in the empirical distributions ---- percentiles calculated by assuming normal distribution of weights within each height group (as assumed by Sundal 1957)

The empirical distributions necessary to reproduce the correct percentiles of the Bergen study are not available However if the shapes of the distributions are believed to be the same as in the Oslo data a recalculation of

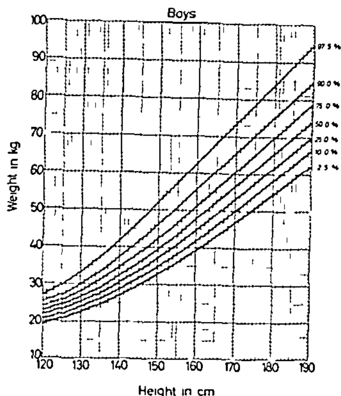


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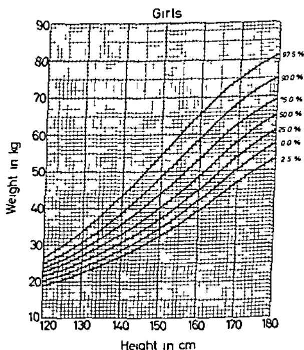


Fig 7 Percentile chart showing weight for height girls Data points calculated by interpolation in the empirical distributions and smoothed visually

from Iowa almost exclusively of northern European descent and from higher social classes are still in use (4) Compared with these Oslo children today are as much as 5 cm (girls) and 6 cm (boys) taller at age eighteen Still for ages below thirteen the data are nearly identical so that the differences evolve during and after puberty To find a stature at age eighteen corresponding to the Iowa data from 1946 we must go back to Oslo measurements from 1975

The percentiles by Tanner (10) which are extensively used are based on measurements on London school children in 1959 (6) and for ages above fifteen on longitudinal data The Oslo percentiles for stature are for girls 3–4 cm above gradually increasing with age for boys 3–5½ cm above The London percentiles do not include weight for height data but our comparable height for age data which for younger girls are somewhat above Tanner s and from age fifteen are identical with his show that Oslo girls in 1970 have less weight for height most pronounced after puberty For boys our data on weight for age are clearly above Tanner s corresponding to the greater body length for age

Swedish data by Broman Dahlberg & Lichtenstein from the late 1930s (1) also show a somewhat smaller stature for age than our data even if their sample is biased (2) The differences increase gradually from 1 cm at age 10 to 3 cm at age 18 both for girls and boys Height/weight relations are almost identical and indicate that Norwegian and Swedish children resemble each other more closely than they resemble British children The newly published Swedish diagrams (2) show close agreement for stature with our Oslo data up to age 10 thereafter the Swedish children are shorter with an increasing difference with increasing age up to 1½ cm at thirteen years whereto data are available

These findings confirm the observations by Meredith (3) in a recent extensive review of growth studies from the period 1950–60 that Norwegian (Oslo) school children have reached a stature higher than any found in comparable studies from other parts of the world

ACKNOWLEDGEMENT

Thanks are due to Oslo City Health Department for financial support and to the school health nurses for their valuable assistance

REFERENCES

1 Broman B Dahlberg G & Lichtenstein A Height and weight during growth *Acta Paediatr Scand 30* 1 1942
2 Engstrom I Karlberg P Klackenberg C Klackenberg Larsson I Lichtenstein H Svennberg I & Taranger J Tillvaxtdiagram for langd vikt och huvudomfång från fodelsen till 18 år *Lakartidn 70* 2960 1973
3 Meredith H V Body size of contemporary youth in different parts of the world *Monogr Soc Res Child Dev* Serial 131 *34* No 7 1969
4 Nelson W E (ed) *Textbook of paediatrics* Saunders Philadelphia 1969 9th ed
5 Schiotz C Physical development of children and young people during the age of 7 to 18–20 years An investigation of 28700 pupils at public (elementary) and higher (secondary) schools in Christiania *Skr norske vidensk akademi I Mat Naturv Kl* No 4 1923
6 Scott J A *Report on the heights and weights (and other measurements) of school pupils in the county of London in 1959* London County Council London 1961
7 *Statistical yearbook of Norway* Central Bureau of Statistics Oslo 1956 and 1973
8 Stuart H C & Meredith H V Use of body measurements in the school health program *Am J Public Health 36* 1365 1946
9 Sundal A *The norms for height (length) and weight in healthy Norwegian children from birth to 15 years of age* Universitetet i Bergen Årbok 1957 Medisinsk rekke No 1
10 Tanner J M Whitehouse R H & Takaishi M Standards from birth to maturity for height weight height velocity and weight velocity British children 1965 *Arch Dis Child* Part I *41* 454 Part II *41* 612 1966
11 Udjus L G *Anthropological changes in Norwegian men in the twentieth century* Oslo University Press Oslo 1964

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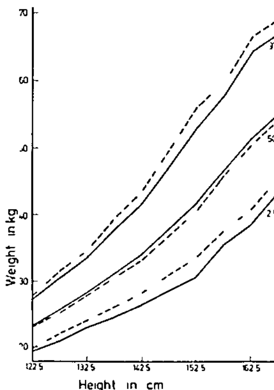


Fig 11 Weight for height percentiles girls Bergen 1956 — percentiles calculated by assuming normal distribution of weights within each height group (plotted from Sundal 1957 p 14 table 4) percentiles calculated from means and standard deviations published by Sundal assuming a distribution of weights within each height group identical in shape to the Oslo data

the corresponding percentiles can be performed (Fig 11) using the available information with regard to the mean values and the standard deviation that is implicit in Sundal s published percentiles (9 table 4 p 14) This transformation shows that the methodological bias involved in Sundals data can amount to a considerable displacement of the percentiles $^1/_2$–1 kg for the medium and up to 1–2$^1/_2$ kg for the extreme percentiles

Using the transformed 1956 data from Bergen a comparison is made with the weight distributions for Oslo children in 1970 in Fig 12 The median weights are seen to be from ½–1 kg higher, the 97 5 percentile from ¾–4 kg higher, for the Oslo girls Similar results are obtained with regard to the boys percentiles The considerable discrepancies between Sundal s and our percentiles for height–weight are thus due partly to methodological causes but also to real differences in body weight

Results with regard to heights can be compared direct The Oslo children in 1970 show a gradually increasing lead in stature for girls from 1 5 to 3 cm for boys from 1 to 4 5 cm through the age range 7–16 years compared with the Bergen children These differences in stature are not only due to a secular change but also reflect geographical variations within Norway Oslo school children measured in 1955 (unpublished results) were taller than the Bergen children by 1–2 cm Differences in stature between military conscripts from the two cities have increased during this period from 0 42 cm (178 11–177 69) in 1955 to 1 8 cm (180 2–178 4) in 1972 (7) There is thus reason to suspect that the previously demonstrated differences in stature between school children in Oslo and Bergen must have *increased* over the last two decades

Stuart & Meredith s percentiles from 1946 (8) based on measurements on school children

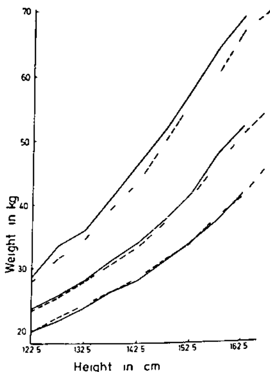


Fig 12 Weight for height percentiles girls Bergen 1956 and Oslo 1970 — empirical percentiles from Oslo 1970 (as solid line in Fig 10) transformed percentiles from Bergen 1956 (as broken line in Fig 11)

Table 1 *The results of bladder washout and relation to laboratory and roentgenological findings*

Result of washout	No of pats	Negative culture the day after the washout	Increased antibody titres[a] Serum unreduced[b]	Serum reduced[c]	ESR (>20 mm/h)	CRP (>10 μg/ml)	Renal concentrating capacity (<814 mOsm/l)	WBC/mm³ urine (>50)	Parenchymal reduction and reflux	Reflux without parenchymal reduction
High infection	12	2	6	4	1	1	1	4	5	2
Indecisive	4	1	0	0	1	1	1	2	2	0
Low infection	44	31	15	7	1	2	0	12	2	2

[a] Against the *E coli* O antigens 1 2 4 6 7 8 18 and 75
[b] Unreduced >7 titre steps ($\log_2$)
[c] Reduced >3 titre steps ($\log_2$)

course and prognosis of ABU in school girls we wanted to get further information on the site of the infection by using a bladder washout test in comparison with serum antibody titration determination of CRP ESR and renal concentrating capacity as well as roentgenological examination We also wanted to consider whether or not earlier noted changes of surface structures in strains causing asymptomatic compared to symptomatic UTI (15 20) could be related to different outcome of the diagnostic parameters employed

MATERIAL AND METHODS

The patients were school girls (7–15 years old) with ABU They represent the first 60 diagnosed with ABU in a series of 116 patients earlier described (14) The patients had been detected without any obvious symptoms in a school screening program by two positive nitrite tests verified by two urine cultures with ≥100 000 bacteria/mm³ The clinical examination did not reveal signs of other infections A history of earlier febrile UTI was obtained in 3 of 13 girls with parenchymal reduction or reflux while one had had an afebrile UTI One of the 47 girls without these roentgenological changes had had pyelonephritis while 10 had been treated for an afebrile UTI

All 60 patients had *E coli* in the urine and the O group distribution as well as the sensitivity to the bactericidal effect of normal serum of the bacteria were the same as for the total group of the 116 patients earlier described (15) The results of the bactericidal test were rated as earlier according to a scale where 0 indicated <50% of the bacteria killed 1 indicated 51–90% killed 2 indicated 91–99% killed and 3 indicated >99% killed (15 20)

The *renal concentrating capacity* was estimated by freeze point reduction in two consecutive urine samples obtained at home after fluid deprivation for at least 15 hours The highest osmolality was noted The age related normal values of Winberg were used as reference (22) If the concentrating capacity was <814 mOsm/l the test was repeated within 2 weeks A second test was performed in 6 of the 60 girls *Leukocytes in uncentrifuged urine* were counted in a Fuchs Rosenthal chamber A count of >50 white blood cells/mm³ was regarded pathologic (1)

The *washout procedure* has been presented earlier (10) and was performed essentially as described by Fairley et al (4) Growth of 1 000 bacteria per ml in one of the three final urine specimens taken every 20 minutes after the washout was chosen as the lowest value consistent with an infection above the ureterovesicular junction ("high infection") providing this represented at least a fivefold increase above the number of bacteria in the washout specimen Growth of 100 bacteria per ml in the final urine specimens was chosen as the highest value accepted to indicate an infection under the ureterovesicular junction ("low infection") Urine for culture was obtained one day after the washout test.

Acta Pædiatr Scand 64 574–580 1975

ASYMPTOMATIC BACTERIURIA IN SCHOOL GIRLS

IV *Difficulties of Level Diagnosis and the Possible Relation to the Character of Infecting Bacteria*

U LINDBERG U JODAL L Å HANSON and B KAIJSER

From the Department of Paediatrics and the Departments of Immunology and Clinical Bacteriology Institute of Medical Microbiology University of Goteborg Goteborg Sweden

ABSTRACT Lindberg U Jodal, U Hanson L Å and Kaijser B (Department of Paediatrics and Departments of Immunology and Clinical Bacteriology Institute of Medical Microbiology University of Goteborg Goteborg Sweden) Asymptomatic bacteriuria in school girls IV Difficulties of level diagnosis and the possible relation to the character of infecting bacteria Acta Paediatr Scand, 64 574 1975.—As no method for localization of urinary tract infection has been shown to be absolutely reliable six methods have been run parallelly in a study of 60 school girls with asymptomatic bacteriuria caused by E. coli A poor correlation was obtained between high infection indicated by bladder washout test and abnormal findings of C reactive protein sedimentation rate antibody titres or renal concentrating capacity while findings of parenchymal reduction on the pyelogram and reflux on the urethrocystogram were often found within this group The low frequency of abnormal findings of C reactive protein sedimentation rate and renal concentrating capacity in girls with a high infection may be explained by the finding that the ABU patients were infected with changed E coli strains probably not able to cause the usual inflammatory reactions The patients bacteria were found to be less efficient in providing antigen for antibody determinations than were standard strains of same 0 group, indicating a difference between strains from ABU patients and standard strains. From the battery of tests used it seemed that most of the girls had a bladder infection All tests were normal in 48% of the patients while at least three of the methods were abnormal in 12%

KEY WORDS Asymptomatic bacteriuria, school girls level diagnosis bladder washout C reactive protein sedimentation rate renal concentrating capacity E coli immunology

The site of the infection is an important factor in the evaluation of the course and prognosis of patients with urinary tract infection (UTI) since patients with bacteriuria of renal origin are probably at risk of developing the renal parenchymal reduction seen in some of these patients (2 7, 17 24) Symptoms like fever loin pain and kidney tenderness suggest an attack of pyelonephritis while frequency and burning without fever indicate cystitis In asymptomatic bacteriuria (ABU) obtaining a proper level diagnosis is more difficult Laboratory findings of increased erythrocyte sedimentation rate (ESR) and C reactive protein (CRP) or decreased renal concentrating capacity which are clearly correlated to the diagnosis of symptomatic pyelonephritis (10) are not often found in ABU patients (14)

As a part of a longitudinal study of the

This study was supported by grants from the first of May Flower Campaign The Faculty of Medicine University of Goteborg the Swedish Medical Research Council (16X 215) and from the Insurance Company Förenade Liv Sweden

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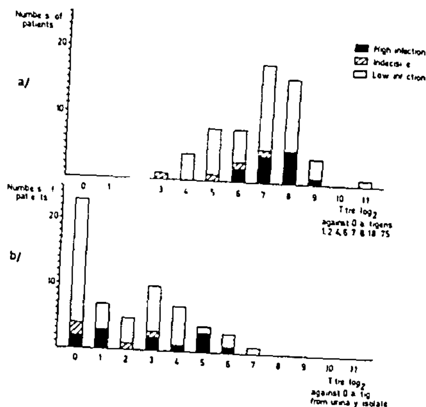


Fig 1 The antibody titres against (*a*) an 0 antigen pool and (*b*) the antigen from the urinary isolate in unreduced serum of 60 school girls with ABU correlated to bladder washout test findings The dotted line indicates the 95th percentile of titres of healthy controls

Blood samples were obtained and analysed for *micro sedimentation rate* (ESR) *C reactive protein* (CRP) (19) and serum antibody titres against *E coli* 0 antigen An ESR>20 mm/h and CRP>10 μg/ml were considered abnormal (10) The *E coli antibody determinations* were done by indirect hemagglutination as earlier described (23) For sensitization was used the crude antigen prepared from the strain of the patient (homologous antigen) the 0 antigen from a standard strain of the same 0 group as the infecting strain and a pool of 8 common *E coli* 0 antigens (0 groups 1 2 4 6 7 8 18 and 75) Treatment of serum with fresh 2 mercaptoethanol (ME) was performed for measurement of the content of reduction resistant antibodies (mainly IgG antibodies) (16) The antibody titres were expressed as $\log_2$ The upper limit of normal titres against the 0 antigen pool for this age group was 7 2 fold dilution steps for unreduced and 3 2 fold dilution steps for reduced serum (8)

Intravenous pyelography (IVP) and *micturition cystourethography* (MCU) were performed in all girls as earlier described (14) For *statistical* evaluation the chi square and the Wilcoxon test for pair differences were employed

RESULTS

A high infection was found in 12 girls (20%) by the washout test and a low infection in 44 girls (73%) while the values were indecisive (i e it was impossible to distinguish a low from a high infection) in 4 (7%) of the 60 patients (Table 1) Thirty one of the 44 patients with a low infection had a negative urine culture the day after the washout test while ≥100000 bacteria/ml urine were found in 10 of the 12 girls with a high infection and in 3 of the 4 girls with indecisive values Only one patient with a high infection had increased ESR (40 mm/h) and CRP (20 μg/ml) and only one had a lowered renal concentrating capacity (764 mOsm/l) Among the patients with a low infection one had increased ESR (31 mm/h) and two had increased CRP (21 and 39 μg/ml) but none of these had renal concentrating capacity <814 mOsm/l Among the four patients with indecisive values in the washout test one had increased CRP (37 μg/ml) and ESR (30 mm/l) as well as lowered renal concentrating capacity (743 mOsm/l)

Pyuria was found in 4 of the 12 girls with high infection in 12 of the 44 girls with low infection and in 2 of the 4 patients with indecisive values in the washout test Pyuria was not more common ($p>0\ 05$) in girls with high infection than in those with low infection

Parenchymal reduction and reflux was found in 9 patients and reflux without parenchymal reduction in 4 patients Seven of the 12 girls with high infection had reflux

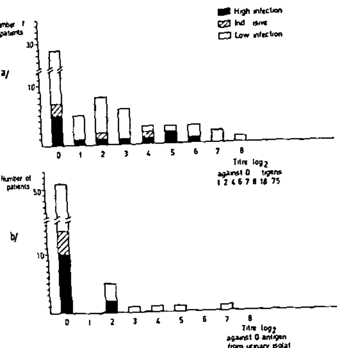


Fig 2 The antibody titres against (*a*) an 0 antigen pool and (*b*) the antigen from the urinary isolate in reduced serum of 60 school girls with ABU correlated to bladder washout test findings The dotted line indicates the 95th percentile of titres of healthy controls

Five of these 7 children had also parenchymal reduction (Table 1) In contrast 4 of the 44 girls with a low infection had reflux and 2 of these 4 had also parenchymal reduction The findings of parenchymal reduction on the pyelogram and reflux on the urethrocystogram were significantly more common ($p<0.01$) in patients with a high infection than in those with a low infection Two of the 4 patients with indecisive washout values had parenchymal reduction with reflux (Table 1)

Twenty six of the 60 patients (43%) had antibody titres against a pool of the 0 antigens 1 2 4 6 7 8 18 and 75 in unreduced or reduced sera increased above the 95th percentile of age matched controls (8) Elevated antibody titres were found in 8 (6 in unreduced and 4 in reduced sera) of the 12 patients with a high infection and in 17 (15 in unreduced and 7 in reduced sera) of the 44 patients with a low infection according to the bladder washout test (Table 1 and Figs 1*a*

Table 2 *The antibody titres against an 0 antigen pool in six of 60 ABU girls with microsedimentation rate >20 mm/h CRP>10 µg/ml or renal concentrating capacity <814 mOsm/l*

Patient	Antibody titres (log₂) in serum		*E coli* strain isolated from the urine	Microsedimentation rate (mm/h)	CRP (µg/ml)	Renal concentrating capacity (mOsm/l)
	Unreduced	Reduced				
M A	8	5	01	40	20	969
S K	6	0	01	31	0	1 085
E K	11	8	0?	9	39	974
A L	5	0	SA*	30	37	743
K L	7	4	SA*	9	0	764
I N	9	3	SA*	18	21	1 127

Parenchymal reduction
* Spontaneously agglutinating

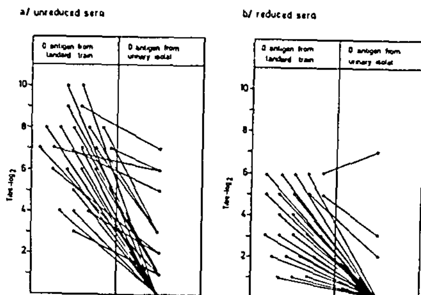


Fig 3 Antibody titres against 0 antigen from the urinary isolate and 0 antigen from a standard strain of the same 0 group in 24 of the 60 girls with ABU (*a*) unreduced sera (*b*) reduced sera

and 2*a*) This was not a significant difference ($p>0\ 05$) Three of the 4 girls with increased CRP one of the 3 girls with ESR>20 mm/h and one of the 2 girls with concentrating capacity <814 mOsm/l had elevated antibody titres against the 0 antigen pool in unreduced or reduced serum (Table 2)

Elevated antibody titres in unreduced serum against the 0 antigen pool were found in 4 of the 9 patients with parencymal reduction and in 17 of the 51 girls without this change This was not significantly different ($p>0\ 05$) But in reduced serum 5 of the 9 girls with parenchymal reduction and 7 of the 51 patients without this change had elevated titres and this difference was significant ($p<0\ 01$)

Antibody titres in unreduced and reduced sera against the crude 0 antigen from the urinary isolate (homologous antigen) from the 60 patients were compared with the titres against the pool of the 0 antigens 1 2 4 6 7 8 18 and 75 (Figs 1*a* and *b* and 2*a* and *b*) No patient had >7 2 fold titre steps in unreduced serum while 3 patients had >3 2 fold titre steps in reduced serum against 0 antigen from the urinary isolate These 3 girls had a "low infection" in the washout test The significant difference in antibody levels ($p<0\ 01$) found when the patients own isolate and when a standard homologous strain were used for sensitization are illustrated for 24 of the 60 girls in Figs 3*a* and *b* These girls had *E coli* strains which were possible to type with available antisera and their bacteria were more sensitive (mean rating value 2 1) to the bactericidal effect of normal serum than the standard strains which were all resistant (rating 0)

The *E coli* strains isolated from patients with a "high infection" or from patients with increased CRP ESR antibody titres or decreased renal concentrating capacity had the same 0 group distribution and sensitivity to the bactericidal effect of normal serum as the bacteria from the rest of the patients

At least three of the laboratory tests (bladder washout antibody titres against the 0 antigen pool CRP, ESR renal concentrating capacity and roentgenological examination) were found abnormal in 7 of the 60 girls (12%) Two tests were found abnormal in 9 girls (15%) one test abnormal in 15 girls (25%) while 29 girls (48%) had no abnormal tests

DISCUSSION

The reliability of the methods (determination of CRP sedimentation rate renal concentrating capacity antibody titration bladder washout and radiological investigation) used in the present study for level diagnosis of UTI has previously been tested in patients with symp

tomatic pyelonephritis and cystitis (10) It was found that of the individual techniques the highest reliability was obtained with determination of CRP followed by antibody titres ESR and renal concentrating capacity when tested against the clinical criteria for level diagnosis while the results from a bladder washout test were more indecisive At least three of the methods including radiological investigation showed pathological findings in patients with symptomatic pyelonephritis

The same tests used in patients with ABU disclosed the difficulties in level diagnosis in this patient group A correlation was obtained between high infection in the bladder washout test and findings of parenchymal reduction on the pyelogram and reflux on the urethrocystogram changes which do not necessarily relate to the actual infection But the correlation to decreased renal concentrating capacity or increased CRP ESR and antibody titres—all tests of the present infection—was poor Thus in most of the patients with a high infection according to the bladder washout test it was not possible to decide if the bacteriuria was of renal origin or if bladder urine retained in the refluxing ureters was emptied into the bladder after washing giving values indicative of renal infection

Values suggesting low infection were obtained in one girl with parenchymal reduction reflux increased CRP and elevated antibody titres and indecisive values were obtained in one girl with parenchymal reduction increased CRP and sedimentation rate as well as decreased renal concentrating capacity This possibly illustrates intermittent discharge of bacteria from the kidney Kass & Zinner (12) have pointed out that a constant outflow of bacteria into the renal pelvis is required if counting of bacteria in ureteric urine is to be used as an indication of kidney infection but that this does not always occur (3 5)

In earlier works we observed that the bacteria isolated from patients with ABU were more sensitive to the bactericidal activity of normal human serum than bacteria from patients with symptomatic infections (15 20) The strains from ABU patients were also often spontaneously nonspecifically agglutinating indicating loss of carbohydrate components from the lipopolysaccharide structure of the bacteria These changes were further substantiated in the present study by the findings that also the ABU strains which could be O grouped were a less potent source of O antigen than the standard strains of the same O group This finding agrees with those of Michael & Landy (18) who found that there was a difference of related strains to coat erythrocytes for agglutination The strains resistant to the bactericidal effect of normal serum were a better source of somatic antigen than their sensitive counterparts

It is possible that these changes of the surface structure of the bacteria make them less efficient in inducing inflammatory changes in an infected kidney explaining the low frequency of lowered renal concentrating capacity and of increased ESR or CRP in the ABU patients with suggestions of a high infection On the other hand it is possible that the elevated antibody titres found in some patients with a low infection may not indicate a renal engagement but a long lasting bladder infection

Obviously there is no simple or definite way presently available to secure the level diagnosis in ABU Whether or not the recently reported technique for localization of a renal infection by detection of antibody coated bacteria in urine sediment (11 21) is reliable in ABU patients where a local production of antibodies probably occurs (9) must be further evaluated However preliminary data indicate that antibody coated bacteria are also present in some ABU patients with a low infection according to the bladder washout test (16)

REFERENCES

1 Braude H Forfar J O Gould J C & McLeod J W Cell and bacterial counts in the urine of normal infants and children *Br Med J* IV 697 1967

2 Davies A G McLachlan M S F & Asscher A W Progressive kidney damage after non obstructive urinary tract infection *Br Med J IV* 406 1972
3 Fairley K F & Butler H M Sterile pyuria as a manifestation of occult bacterial pyelonephritis with special reference to intermittent bacteriuria *In* P Kincaid Smith & K F Fairley (eds) *Renal infection and renal scarring* Mercedes Melbourne 1971 p 51
4 Fairley K G Grounds A D Carson N E Laird L C Gutch R C McCallum P H G Leighton P Sleeman R L & O Keefe C M Site of infection in acute urinary tract infection in general practice *Lancet II* 615 1971
5 Geisinger J F Intermittance of pyuria at the level of the renal papillae *J Urol 25* 649 1931
6 Hanson L Å Holmgren J Jodal U & Kaijser B Characterization of precipitating antibodies to *E coli* 0 antigen in infants and children with acute pyelonephritis *Clin Exp Immunol 8* 573 1971
7 Hodson C J & Wilson S Natural history of chronic pyelonephritis scarring *Br Med J II* 191 1965
8 Jodal H The immune response to urinary tract infections in childhood I Serological diagnosis of primary symptomatic infection in girls by indirect hemagglutination *Acta Paediatr Scand 64* 96 1975
9 Jodal U Ahlstedt S Carlsson B Hanson L Å Lindberg U & Sohl A Local antibodies in childhood urinary tract infection A preliminary study *Int Arch Allergy Appl Immunol 47* 537 1974
10 Jodal U Lindberg U & Lincoln K Level diagnosis of symptomatic urinary tract infections in childhood *Acta Paediatr Scand 64* 201 1975
11 Jones S R Smith J W & Sanford J P Localization of urinary tract infections by detection of antibody coated bacteria in urine sediment *N Engl J Med 290* 591 1974
12 Kass E H & Zinner S H Bacteriuria and renal disease *J Infect Dis 120* 27 1969
13 Lincoln K Lidin Jansson G & Winberg J Resistant urinary infections resulting from changes in resistance pattern of faecal flora induced by sulphocemide and hospital environment *Br Med J III* 305 1970
14 Lindberg U Claesson I Hanson L Å & Jodal U Asymptomatic bacteriuria in school girls I Clinical and laboratory findings *Acta Paediatr Scand 64* 425 1975
15 Lindberg U Hanson L Å Jodal U Lidin Jansson G Lincoln K & Olling S Asymptomatic bacteriuria in school girls II Differences in *Escherichia coli* causing asymptomatic and symptomatic bacteriuria *Acta Paediatr Scand 64* 437 1975
16 Lindberg U & Kaijser B Unpublished data
17 Lindblad B S & Ekengren K The long term prognosis of nonobstructive urinary tract infection in infancy and childhood after the advent of sulphonamide *Acta Paediatr Scand 58* 25 1969
18 Michael J G & Landy M Endotoxic properties of gram negative bacteria and their susceptibility to the lethal effect of normal serum *J Infect Dis 111* 90 1961
19 Nilsson L Å Comparative testing of precipitation method for quantitation of C reactive protein *Acta Pathol Microbiol Scand 73* 129 1968
20 Olling S Hanson L Å Holmgren J Jodal U Lincoln K & Lindberg U The bactericidal effect of normal serum on *E coli* strains from normals and from patients with urinary tract infections *Infection 1* 24 1973
21 Thomas V Shelokov A & Forland M Antibody coated bacteria in the urine and the site of urinary tract infection *N Engl J Med 290* 598 1974
22 Winberg J Determination of renal concentrating capacity in infants and children without renal disease *Acta Paediatr Scand 48* 318 1959
23 Winberg J Andersen H J Hanson L Å & Lincoln K Studies of urinary tract infections in infancy and childhood I Antibody response in different types of urinary tract infections caused by coliform bacteria *Br Med J II* 524 1963
24 Winberg J Berstrom T & Hanson L Å Neue Gesichtspunkte zur Harnwegsinfektion mit *E coli* *Monatsschr Kinderheilkd 119* 506 1971

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THE SEX VARIABLE IN CHILDHOOD URINARY TRACT INFECTION

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ABSTRACT Bahna S L and Torp K H (Department of Paediatrics County Hospital of Troms Tromso Norway) The sex variable in childhood urinary tract infection Acta Paediatr Scand 64 581 1975.—*Sex differences in childhood urinary tract infection have been looked for* by reviewing the medical records of all patients who were admitted to one medical centre during a certain time period There were 240 patients 26 males and 214 females all under 14 years of age The disease in boys—as compared with that in girls—was *found to be characterized* by an earlier onset a shorter delay in diagnosis and a shorter duration but a higher frequency of malformations a greater number of rehospitalizations and a greater need for surgical intervention Proteus infection was found more frequently in boys while Enterococcus and *Staphylococcus were more frequent in girls* The initial symptomatology did not show any significant sex difference except in late childhood where the non specific symptoms were more common in females No sex difference was noted with respect to the frequency of vesico-ureteric reflux or of bacteriuria without pyuria It seems *that the sex variable in this disease* is worth considering in dealing with individual patients as well as in presenting data on a series of patients

KEY WORDS Urinary tract infection in children sex differences

Urinary tract infection (UTI) in children is now well recognized as a common condition which is potentially serious but often over looked For a better understanding of this dis ease an attempt has been made to classify the patients into various subgroups according to variables such as age sex recurrence radiologic findings and pathogens Although the female preponderance among children with UTI is consistently noted yet in only a few studies has consideration been given to sex as a relevant variable Even in those studies with the exception of a recent one (?) comparisons between male and female pa tients are subsidiary and cover few features of the disease

The accumulating information from various reports suggests that UTI in boys is different in one feature or another from that in girls (2 7 14–16) Definite conclusions however are not yet established with respect to most of the reported differences particularly the infre quently noted ones For this reason studies dealing with sex differences in childhood UTI are greatly needed

In the present study medical records of a series of children who were hospitalized for UTI have been reviewed The purpose was to explore possible sex differences which might be of importance in management of the dis ease The whole series is taken from one de partment and has been treated with the same policy

MATERIAL AND METHODS

Of the admissions during 1968 through 1970 in the De partment of Paediatrics County Hospital of Troms 240 had UTI They comprise 26 boys and 214 girls all under

Table 1 *Distribution of 26 boys and 214 girls by age at onset of urinary tract infection*

Sex		Total	Age at onset (years) <1	1–2	3–4	5–6	7–9	10–13	Mean
Boys	*n*	26	12	8	2	1	1	2	2 6
	%	100	46 2	30 8	7 7	3 8	3 8	7 7	
Girls	*n*	214	21	40	88	38	23	4	4 3
	%	100	9 8	18 7	41 1	17 8	10 7	1 9	

14 years of age Some of these children had been previously admitted into the same department for the same reason It is pertinent to mention that this is the only department of paediatrics in the county of Troms a point which greatly minimizes the possibility of selection bias from the population

When first admitted all patients were symptomatic and the diagnosis was based mainly on a significant bacterial growth (colony count of at least 100 000 organisms per ml) in a fresh cleanly collected specimen of urine The diagnosis in 25 girls already receiving chemotherapy at the time of presentation and showing low colony counts was based on an unequivocal clinical history of UTI Urinalysis was done for all patients more than 10 leucocytes per high power field of urine sediment has been designated as pyuria (5 13) Both intravenous pyelography and retrograde cystography were performed in 6 boys and 108 girls I V pyelography alone in 15 boys and 73 girls and cystography alone in 2 boys and 8 girls

Comparisons between males and females comprised age at onset initial symptom interval between onset and initial diagnosis causative organism *at first admission* radiologic findings *at first admission* duration of illness number of readmissions and surgical intervention

Age at onset is taken as the age when the initial symptom first appeared The initial symptom is selected as the main symptom mentioned by the parents or the child as the first to appear in the chain of events which eventually proved to be due to UTI The duration of illness is calculated from the date of onset till the date of last treatment The *readmissions* are those for UTI and include also the referrals to other hospitals

The findings are statistically evaluated by application of the chi square test with 1 degree of freedom (d f) in comparing frequencies the *t* test with n_1+n_2-2 d f in comparing two arithmetic means and the *t* test with $n-2$ d f in testing the coefficient of correlation (*r*) An observed difference has been considered statistically significant if *p* was less than 0 05

RESULTS

Age at onset (Table 1)

The age at onset was under 1 year in about one half of the boys but in only one tenth of the girls with an average of 2 6 years in boys and 4 3 years in girls a difference which is statistically significant In boys the peak of onset was in the first year of life while in girls it occurred around the age of 4 (Fig 1)

Initial symptom (Table 2)

Comparing male and female patients at various age groups with regard to initial symptoms

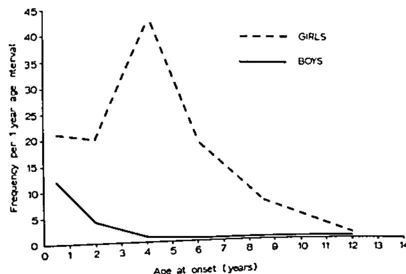


Fig 1 Age at onset of urinary tract infection in boys and in girls

Table 2 *Initial symptom of urinatry tract infection in boys and girls at various age groups*

Age at onset (yr)		Total		Failure to thrive[b]		Fever of unk origin		Urinary symptoms		Rank order of initial symptoms
		n	%	n	%	n	%	n	%	
<2	♂	15	100	8	53 3	7	46 7	–	–	Failure to thrive fever
	♀	6	100	10	38 5	16	61 5	–	–	Fever failure to thrive
2–4	♂	4	100	1	25 0	1	25 0	2	50 0	Dysuria failure to thrive fever
	♀	114	100	7	6 1	17	14 9	90	79 0	Dysuria frequency fever enuresis failure to thrive
5–13	♂	4	100	–	–	–	–	4	100 0	Dysuria enuresis frequency
	♀	58	100	8	13 8	13	22 4	37	63 8	Dysuria enuresis fever frequency failure to thrive

Excluding 19 (3 boys & 16 girls) in whom one initial symptom was not clearly reported
[b] Including non specific symptoms such as weight loss poor appetite vomiting or vague abdominal pain
Dysuria frequency or enuresis in children who had been previously toilet trained

revealed no significant differences except at age 5 to 13 years where urinary symptoms were more common in boys while failure to thrive and fever of unknown origin were more common in girls

Interval between onset and initial diagnosis

On the average this interval was shorter in boys (1 7 months) than in girls (2 4 months) however the difference is not statistically significant It was noted that all the 12 boys with onset during the first year of life were diagnosed before reaching 1 year of age while the corresponding figures in girls are 12 out of 21

Causative organism (Table 3)

Escherichia coli was the main pathogen of the urinary tract in boys and in girls with no significant difference A sex difference is observed with respect to Proteus which was significantly more frequent in males and with respect to Enterococcus and Staphylococcus which were found only in females

Bacteriuria without pyuria showed almost the same frequency in boys (26 9%) and girls (25 6%)

Radiologic findings

Intravenous pyelography revealed abnormalities in 5 out of 21 boys (23 8%) and in 26 out of 181 girls (14 4%) however this difference is not statistically significant Congenital malformations (double collecting systems aberrant blood vessel cyst tumour horseshoe kidney or diverticula) were

Table 3 *Causative organisms of urinary tract infection in boys and in girls*

	Boys (n=26)	Girls (n=189)
Esch coli		
n	14	114
%	53 8	60 3
Proteus		
n	6	19
%	23 1	10 1
Paracolon		
n	2	18
%	7 7	9 5
Aerobacter		
n	1	13
%	3 8	6 9
Enterococcus		
n	–	10
%	–	5 3
Staphylococcus		
n	–	3
%	–	1 6
Micrococcus		
n	–	1
%	–	0 5
Mixed		
n	3	11
%	11 5	5 8

Excluding 25 who were receiving chemotherapy at the time of presentation

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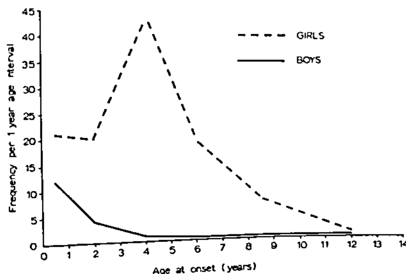


Fig 1 Age at onset of urinary tract infection in boys and in girls

tation in 3 boys (11 5%) and 11 girls (5 1%) nephrectomy in 3 boys (11 5%) and 2 girls (0 9%) and ureteroileostomy in 1 boy

DISCUSSION

The present findings indicate that childhood UTI in males is different in many respects from that in females Before proceeding into the discussion it should be realized that the small figures resulting from subclassifications did not allow more detailed comparisons which might reveal further differences

The literature did not provide consistent information on the peak of onset of UTI in children In a mixed material Segura et al (12) found a peak under 1 year of age followed by a larger one at age 2 to 4 In girls Cohen (5) found the peak at 3 years of age On the other hand Smellie et al (13) noted the peak to be in the first year of life in each sex The present study shows that each sex has its own peak the one for boys is in the first year of life and that for girls is around the age of 4 years This finding may point to the existence of two different aetiological mechanisms underlying these two peaks It is generally believed that UTI during early infancy and particularly in males is mainly haematogenous (3 8) while at a later age it is mainly ascending—a route which is much easier in girls than in boys (4 6 12) The high frequency of urologic malformations in boys could not account for the early onset of UTI in them a finding which confirms that of O Doherty (11)

Under the age of 5 years the initial symptom did not differ significantly between boys and girls In older children however urinary symptoms were more common in boys while the non specific ones were more common in girls This finding does not completely conform with the report of Bergstrom (2) that at the age of 1 to 16 years fever is more common in female patients

The delay in diagnosis of UTI particularly in children with urologic malformations was noted to be shorter in males than in females The reverse would be expected on the basis that in the majority of boys—but in a minority of girls—the age at onset was under 3 years where the manifestations are mainly non specific It may be that UTI in girls is presented later or misdiagnosed more often than in boys

There was no sex difference in the rank order of the four most common causative organisms namely *E coli* Proteus Paracolon Aerobacter Similar rank orders have been reported by others (6 7 13) Neither was there a sex difference in the frequency of *E coli* infection an observation which conforms with that of Forbes et al (7) but not with that of Bergstrom (2) and Mann (10) The higher frequency of Proteus infection among boys has also been previously reported (2 10) but the contrary was found by Forbes et al (7) The sex difference observed with respect to Enterococcus and Staphylococcus being more frequent in girls was not noted in the series of Bergstrom (2)

A higher frequency of urologic malformations among boys has also been noted by some authors (12 14 15)

Vesico-ureteric reflux however showed approximately the same frequency in boys and girls a finding which is similar to that in other studies (1 7, 14)

The observation of dilatation of the upper urinary tract in 6 girls but in none of the boys is contrary to that of Bergstrom (2) These girls were peculiar in that reflux was demonstrated in all of them and their average delay in diagnosis was much higher than in those without dilatation

The tendency of the duration of illness to be longer in females than in males may be due to a milder and more chronic course of the disease and/or to the easier reinfections through the female urethra

It might be unexpected then to find the average number of readmissions to be higher in boys As the difference was only partly accounted for by the higher frequency of malformations it may be that the disease is

Table 4 *Frequency of urologic malformations in boys and girls discovered by intravenous pyelography according to the interval between onset and diagnosis of urinary tract infection*

Interval between onset and diagnosis	Frequency of malformations	
	Boys	Girls
Less than 1 week	5/13 (38 5%)	14/129 (10 9%)
One week or more	0/8 (0 0%)	5/46 (10 9%)
Unknown	-	0/6
Total	5/21 (23 8%)	19/181 (10.5%)

observed in the 5 boys (23 8%) and 19 girls (10 5%) Dilatation of the upper urinary tract was noted in 6 girls and calcification in 1 girl

Neither in boys nor in girls was the mean age at onset significantly related to the finding of urologic malformations Among the investigated children with onset during the first year of life malformations were found in 2 out of 9 boys and in 1 out of 15 girls

An obvious relationship between the frequency of malformations and the interval between onset and diagnosis was found in boys but not in girls (Table 4) In children with malformations the diagnosis of UTI was established within 1 week from onset in all the 5 boys and in 14 girls between 1 week and 6 months in 4 girls and 4 years in 1 girl

Retrograde cystography revealed abnormalities in 4 out of 8 boys (50 0%) and in 51 out of 116 girls (43 3%) this difference is not statistically significant The abnormality was in the form of vesico ureteric reflux in the 4 boys (50 0%) and 48 girls (41 4%) bladder neck obstruction, diverticulum and calcification, each in 1 girl

It is noteworthy that the 6 girls with dilatation of the upper urinary tract all had vesico ureteric reflux, and their average interval between onset and diagnosis was 6 9 months which is about 3 times as much as in girls without dilatation

Duration of illness

This duration varied from less than a month up to 10 years it was 2 years or more in 38 5% of boys and 57 5% of girls The average was 1 8 years in boys and 2 9 years in girls however this difference is not statistically significant The difference is much greater in patients with normal initial pyelogram where the average duration of illness was 0 6 years for boys and 3 1 years for girls, a difference which is highly significant On the other hand, in children with urologic malformations the average duration of illness did not differ significantly between males (3 9 y) and females (3 4 y)

No significant correlation was found between the duration of illness and the age at onset in the present material r was +0 10 Therefore the sex difference in the duration of illness is not due to the difference in the age at onset Neither is it due to the longer interval between onset and diagnosis in females as it was still noted on comparing boys with girls who had the same delay in diagnosis

Number of readmissions

Rehospitalization for UTI occurred in almost the same proportion in boys (50 0%) and girls (51 4%) Referral to other hospitals with better facilities—mainly for surgical intervention—was significantly more common among boys (34 6%) than among girls (12 1%)

The number of readmissions varied from 1 to 8 with an average of 3 2 in boys and 2 0 in girls a difference which is statistically significant The difference was not appreciably reduced by exclusion of children with urologic malformations the average number of readmissions was 2 9 for boys and 1 9 for girls

In the present material the number of readmissions per capita did not show significant correlation with the duration of illness r was +0 12

Surgical intervention

Surgery was performed in a total of 7 boys (26 9%) and 13 girls (6 1%) ureteral reimplan

Acta Pædiatr Scand 64 587–591 1975

THE CONCENTRATIONS OF TOTAL CORTISOL AND CORTICOSTERONE IN MIXED CORD PLASMA

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ABSTRACT Homoki J Teller W M Tschurtz D and Fazekas A T A (Department of Paediatrics Division of Endocrinology and Metabolism University of Ulm/Donau BRD) The concentrations of total cortisol and corticosterone in mixed cord plasma Acta Paediatr Scand 64 587 1975.—Cortisol and corticosterone were determined in mixed umbilical cord plasma of 43 healthy full term newborns The method consisted of a combined thin layer chromatographic-fluorimetric procedure which proved to be specific and reliable The mean concentration in cord plasma of cortisol was 10 6±4 9 μg/100 ml of corticosterone 1 8±0 8 μg/100 ml The mean ratio cortisol/corticosterone F/B was 6.3±2.5 Neither the duration nor the time of day of delivery appeared to influence the concentration of cortisol or corticosterone in umbilical cord plasma Also there was no significant difference between male and female infants In 18 instances of a pathological course of gestation and/or delivery the mean cortisol level was 9 1±4 7 μg/ml the mean corticosterone level 2.2±9 μg/100 ml The mean F/B ratio was slightly but not significantly decreased (4 2±1 4 μg/100 ml $p>0$ 05) It is speculated that the high corticosterone concentration in umbilical cord plasma reflects a defect in cortisol biosynthesis (17α hydroxylase deficiency) in the newborn compared with later life

KEY WORDS Cortisol corticosterone cord plasma normal newborns, perinatal distress

Only a few studies have appeared concerning the concentrations of total cortisol (F) and corticosterone (B) in mixed human umbilical cord plasma The results varied possibly due to a difference in methods used for determination of these corticosteroids

It therefore appears justified to report our results from simultaneous determination of F and B in mixed human cord plasma The method employed consisted of a combined thin layer chromatographic fluorimetric procedure It proved to be specific and reliable As in the approach of Painter et al (22) the procedural loss was determined in each and every plasma sample extracted by the use of radioactive tracers All steroid values obtained were corrected for their individual losses thus overcoming the variation in yield occurring between samples in a larger series

Dedicated to Professor Dr K Hinsberg Freiburg on the occasion of his eightieth birthday

This work was supported by the Deutsche Forschungsgemeinschaft Bad Godesberg (SFB 87 Project C 3) It forms part of the M D thesis of D Tschurtz at the University of Ulm 1974

SUBJECTS MATERIALS AND METHOD

Subjects and sampling

From 43 full-term normal newborns and 18 patients following pathological conditions of pregnancy and/or delivery (6 cases of caesarean section 5 cases of prematurity 2 cases of estrogen treatment during pregnancy 1 case each of umbilical cord around the neck maternal diabetes maternal hyperthyroidism toxemia of pregnancy and postmaturity) umbilical cord blood was obtained during the day between 7 a m and 5 p m The blood was collected in heparinized tubes and immediately centrifuged The plasma was kept at −28°C until processing

more severe in boys than in girls (9 16) In this series the higher severity of the disease in boys may be indicated by their higher mean number of rehospitalizations rate of referral to other hospitals and rate of surgical intervention

From the present study it seems that sex differences in UTI are worth considering both in research studies and in general practice It appears that reports on childhood UTI would be more informative if they deal with one sex or include sex classifications In practical terms boys with UTI should be especially looked for during infancy and be expected to have higher frequency of urologic malformations Proteus infection severity rehospitalization and need for surgical intervention than girls However the duration of illness should be expected to be longer in females

REFERENCES

1 Baker R Maxted W Maylath J & Shuman I Relation of age sex and infection to reflux Data indicating high spontaneous cure rate in pediatric patients *J Urol 95* 27 1966

2 Bergstrom T Sex differences in childhood urinary tract infection *Arch Dis Child 47* 227 1972

3 Bergstrom T Larson H Lincoln K & Winberg J Studies of urinary tract infections in infancy and childhood XII Eighty consecutive patients with neonatal infection *J Pediatr 80* 858 1972

4 Brumfitt W & Percival A Pathogenesis and laboratory diagnosis of non tuberculous urinary tract infection A review *J Clin Pathol 17* 482 1964

5 Cohen M Urinary tract infections in children I Females aged 2 through 14 first two infections *Pediatrics 50* 271 1972

6 DeLuca F G Fisher J H & Swenson O Review of recurrent urinary tract infections in infancy and early childhood *N Engl J Med* 268 75 1963

7 Forbes P A Drummond K N & Nogrady M B Initial urinary tract infections Observations in children without major radiologic abnormalities *J Pediatr 75* 187 1969

8 Littlewood J M 66 infants with urinary tract infection in first month of life *Arch Dis Child 47* 218 1972

9 Macaulay D & Sutton R N P The prognosis of urinary tract infection in childhood *Lancet II* 1318 1957

10 Mann P G Proteus urinary infections in childhood *J Clin Pathol 25* 551 1972

11 O Doherty N Urinary tract infection in the neonatal period and later infancy *In* F O Grady & W Brumfitt (eds) *Urinary tract infection* Oxford University Press London 1968 p 113

12 Segura J W Kelalis P P Stickler G B & Burke E C Urinary tract infection in children A retrospective study *J Urol 105* 591 1971

13 Smellie J M Hodson C J Edwards D & Normand I C S Clinical and radiological features of urinary infection in childhood *Br Med J* ? 1 ?? 1964

14 Smellie J M & Normand I C S Experience of follow up of children with urinary tract infection *In* F O Grady & W Brumfitt (eds) *Urinary tract infection* Oxford University Press London 1968 p 123

15 Stansfeld J M Clinical observations related to incidence and aetiology of urinary tract infections in children *Br Med J I* 631 1966

16 Steele R E Leadbetter G W & Crawford J D Prognosis of childhood urinary tract infection *N Engl J Med 269* 883 1963

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ABSTRACT Homoki J Teller W M Tschurtz D and Fazekas A T A (Department of Paediatrics Division of Endocrinology and Metabolism University of Ulm/Donau BRD) The concentrations of total cortisol and corticosterone in mixed cord plasma Acta Paediatr Scand 64 587 1975.—Cortisol and corticosterone were determined in mixed umbilical cord plasma of 43 healthy full term newborns. The method consisted of a combined thin layer chromatographic–fluorimetric procedure which proved to be specific and reliable The mean concentration in cord plasma of cortisol was 10 6±4 9 μg/100 ml of corticosterone 1 8±0 8 μg/100 ml The mean ratio cortisol/corticosterone *F/B* was 6.3±2.5 Neither the duration nor the time of day of delivery appeared to influence the concentration of cortisol or corticosterone in umbilical cord plasma Also there was no significant difference between male and female infants In 18 instances of a pathological course of gestation and/or delivery the mean cortisol level was 9 1±4 7 μg/ml the mean corticosterone level 2 2±9 μg/100 ml The mean *F/B* ratio was slightly but not significantly decreased (4 2±1 4 μg/100 ml $p>0\ 05$) It is speculated that the high corticosterone concentration in umbilical cord plasma reflects a defect in cortisol biosynthesis (17α hydroxylase deficiency) in the newborn compared with later life

KEY WORDS Cortisol corticosterone cord plasma normal newborns perinatal distress

Only a few studies have appeared concerning the concentrations of total cortisol (*F*) and corticosterone (*B*) in mixed human umbilical cord plasma The results varied possibly due to a difference in methods used for determination of these corticosteroids

It therefore appears justified to report our results from simultaneous determination of *F* and *B* in mixed human cord plasma The method employed consisted of a combined thin layer chromatographic fluorimetric procedure It proved to be specific and reliable As in the approach of Painter et al (22) the procedural loss was determined in each and every plasma sample extracted by the use of radioactive tracers All steroid values obtained were corrected for their individual losses thus overcoming the variation in yield occurring between samples in a larger series

Dedicated to Professor Dr K Hinsberg, Freiburg on the occasion of his eightieth birthday

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SUBJECTS MATERIALS AND METHOD

Subjects and sampling

From 43 full term normal newborns and 18 patients following pathological conditions of pregnancy and/or delivery (6 cases of caesarean section 5 cases of prematurity 2 cases of estrogen treatment during pregnancy 1 case each of umbilical cord around the neck maternal diabetes maternal hyperthyroidism toxemia of pregnancy and postmaturity) umbilical cord blood was obtained during the day between 7 a m and 5 p m The blood was collected in heparinized tubes and immediately centrifuged The plasma was kept at −28°C until processing

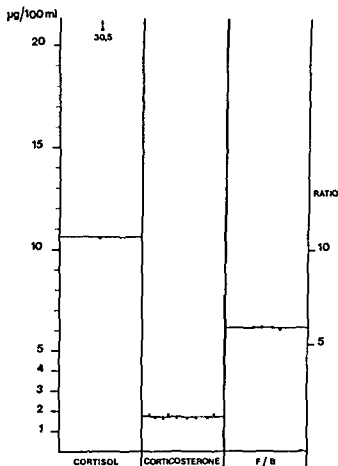


Fig 1 Cortisol (*F*) and corticosterone (*B*) concentrations and *F/B* ratios in mixed umbilical cord plasma of normal full term newborns

Analytical procedure

The method was modified from Ganjam et al (11) To 1–4 ml of plasma 1000 cpm each of 4 ¹⁴C cortisol and 4 ¹⁴C corticosterone were added Bidistilled water was added to bring the volume up to 5 ml The plasma was extracted once with 5 ml of petrol ether to remove lipoid impurities Afterwards it was extracted once with 40 ml of ice-cooled methylenedichloride The watery phase was discarded The extract was washed with 4 ml of 0 1 N sodium chloride and 40 ml of bidistilled water Following the washings the extract was dried down in a rotatory evaporator taken up in 5 drops of chloroform/methanol (1 1) and streakwise applied to silica gel folia (G/Uv 254 thickness 0 25 mm prefabricated by Macherey & Nagel) Along both outer sides a mixture of pure reference steroids was applied The ascending development was performed for 45 minutes at room temperature using the solvent system chloroform/methanol/toluene/water (60 20 120 1) Afterwards the folia were dried The reference compounds were visualized by spraying with a solution of alkaline tetrazolium blue The cortisol and corticosterone areas of the chromatograms were cut with scissors and eluted with a mixture of methylene dichloride/methanol (9 1) and 2 ml of bidistilled water The eluate was dried down in the rotatory evaporator and taken up in 1 ml of methanol 0 2 ml of this solution was counted in the liquid scintillation spectrophotometer (Packard TRI CARB model 3380) 0 8 ml was again evaporated taken up in 2 ml of a mixture of sulphuric acid/methanol (65 35) and vigorously shaken for 1 min

Thirty minutes later the fluorescence of cortisol was determined in a spectral fluorimeter (Fluorispec SF 100 Baird Atomic) (5 ml cuvettes 1 cm pathway primary light 470 nm secondary light 526 nm wave length) 45 minutes after shaking corticosterone was determined in a similar procedure (472 nm primary and 526 nm secondary light)

Calculations

Standard curves were obtained with pure reference compounds Known amounts of labelled and unlabelled cortisol and corticosterone were dissolved in bidistilled water and taken through the entire analytical procedure Following correction by simultaneous measurements of recoveries the resultant curves corresponded exactly to those obtained by direct fluorimetry of increasing amounts of pure cortisol and corticosterone These curves were linear in the range of 0 05–0 4 µg of cortisol and 0 005–0 04 µg of corticosterone

The steroid concentrations in plasma were calculated as follows

$$\text{Steroid concentration [}\mu g/100 \text{ ml plasma]} = \frac{(F + {}^1/_5 F) \times M \times 10000}{F \times V \times R}$$

F=fluorescence of the unknown sample (after subtraction of blank value) M=µg of pure steroid taken directly for fluorescence without extraction F =fluorescence of M V=volume of plasma sample (ml) R=percentage recovery of radioactive tracer added to plasma sample prior to extraction

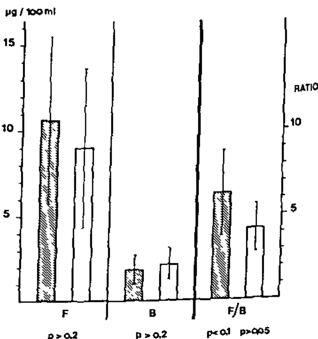


Fig 2 Cortisol (*F*) and corticosterone (*B*) concentrations and *F/B* ratios in mixed umbilical cord plasma following normal and pathological gestation and/or delivery [hatched] Normal full term newborns (n=43) [open] pathological conditions of gestation and/or de

Table 1 *Survey of results reported in the literature of simultaneous cortisol and corticosterone determinations in human plasma*

Authors	Age group	*n*	Cortisol (*μg/100 ml*)	Cortico-sterone (*μg/100 ml*)	Cortisol/ Cortico-sterone	Method
Sweat (1955)	Adults	21	10 8	4 3	2 5	Fluorimetry
Bondy & Upton (1957)	Adults	29	10 2	1 3	7 8	Fluorimetry
McLaughlin et al (1958)	Adults	21	12 4	2 0	6 2	Fluorimetry
Ely et al (1958)	Adults	20	10 9	3 0	3 6	Fluorimetry
Peterson & Pierce (1960)	Adults	20	–	1 1	–	Fluorimetry
Van der Vies (1961)	Adults	5	13 8	5 2	2 6	Fluorimetry
Van der Wal et al (1961)	Adults	8	11 6	3 3	3 5	Fluorimetry
Stewart et al (1961)	Adults	27	9 6	0 4	24	Fluorimetry
De Moor et al (1962)	Adults	673	17 7	3 5	5 0	Fluorimetry
Hillman & Giroud (1965)	Cord blood	17	10 04	–	–	Double isotope dilution
Martin & Martin (1968)	Adults	33	9 6	0 6	16	Fluorimetry
Brorson (1968)	Adults	28	10 3	0 6	17	Fluorimetry
Fraser & James (1968)	Adults	17–29	9 8	0 66	14 8	Double isotope dilution
Schweitzer et al (1969)	Cord blood	7	3 6±1 5	0 3±0 2	12	Double isotope dilution
Teller & Beckmann (1969)	Newborns	14	14 7	6 94	2 35	Fluorimetry
Ferrante (1970)	Adults	–	9 15±2 74	2 13±1 53	4 3	Protein binding
Hamanaka et al (1970)	Men	31	12 8±0 6	0 3±0 1	42 7	Fluorimetry
	Women	24	12 9±0 5	0 4±0 1	32 3	
Huther & Scholz (1970)	Adults	18	14 6±1 36	1 68±0 14	8 6	Fluorimetry
Iturzaeta et al (1970)	Adults	0	11 7±3 7	–	–	Protein binding
	Children	46	10 7±4 8			
Jubiz et al (1970)	Adults	6	16 0±3 0	–	–	Protein binding
Newsome et al (1972)	Adults	11	12 3±0 8	0 4±0 03	30 7	Protein binding
Barnes et al (1972)	Children adolescents	70	17 3±7 8	–	–	Protein binding
This paper (1974)	Cord blood	43	10 6±4 9	1 8±0 8	6 3±2 5	Fluorimetry

Discussion of method

The solvent system used did not separate corticosterone and 11-deoxycortisol The R_F values were 0 4 and 0 38 respectively Since 11-deoxycortisol does not give fluorescence with sulphuric acid under the conditions employed this pitfall in the procedure could be disregarded

Sensitivity

The blank values of the TLC folia were below the lowest limits of detection of individual corticosteroids (cortisol 1 25 μg/100 ml plasma corticosterone 0 125 μg/100 ml plasma) Thus the lowest plasma concentration measured reliably was 2 5 μg/100 ml of cortisol and 0 25 μg/100 ml of corticosterone

The radioactively measured recoveries ranged between 46 and 60% for cortisol and 42–49% for corticosterone All our steroid values reported in this paper were corrected for the procedural losses The intensity of fluorescence of cortisol reached a plateau after 30 min that of corticosterone after 45 min. Thereafter it remained constant for 5 hours in both reactions

Precision and specificity

The coefficent of variation was ±3 7% Because of the pre-extraction with petrol ether and thin layer chromatographic separation the specificity of the determinations can be regarded as quite satisfactory Experiments with mixtures of twelve pure C_{21} steroids proved that only 11-deoxycortisol has a similar R_F as corticosterone however it does not yield fluorescence under conditions used in this study In the cortisol area there was no overlap

RESULTS

In mixed umbilical cord plasma of 43 normal full term newborns the average concentration of cortisol (*F*) was 10 6±4 9 μg/100 ml and the average concentration of corticosterone (*B*) 1 8±0 8 μg/100 ml The mean *F*/*B* ratio amounted to 6 3±2 5 (Fig 1)

No significant differences in steroid concentrations were noted between male and female newborns also time and duration of delivery seemed to have no bearing on the *F* and *B* concentrations in cord plasma

In 18 instances of pathological conditions of pregnancy and/or delivery the following steroid concentrations were obtained (mean ±1 S D)

cortisol	9 0±4 7 μg/100 ml
corticosterone	2 2±0 9 μg/100 ml
F/B ratio	4 2±1 4

These values were not significantly different from the normal values The *F/B* ratio in pathological conditions revealed a tendency towards lower values though the difference from normal values was statistically not significant ($p>0\ 05$) (Fig 2)

DISCUSSION

Several reports on cortisol and corticosterone plasma concentrations have been published (Table 1) Most of them give results obtained in adults They show a rather wide scatter of values The differences can largely be accounted for by the different methods employed In children only scant information is available Our results correspond best to the values reported by Ferrante (8) and Huther & Scholz (15) The former author used protein binding the latter fluorimetry as end point Franks (9) reported the absence of diurnal rhythm of 17 hydroxycorticosteroid plasma concentrations in infants up to the age of one year These results conform to our findings of absent diurnal variations of cortisol and corticosterone in cord plasma at deliveries throughout the day Also the duration of delivery was without influence on the corticosteroid concentrations in cord plasma

The relatively high plasma concentrations of corticosterone and the low *F/B* ratios at birth point towards an increased production of corticosterone during the perinatal period compared with later life One could speculate as Hughes et al (16) did that in the human newborn 17 hydroxylation is less efficient and possibly not yet fully developed

In adults undergoing situations of surgical stress elevated concentrations of corticosterone and lowered *F/B* ratios were described by Hamanaka et al (12) The response in children and adults of *F/B* ratios to ACTH stimulation remains to be further elucidated

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REFERENCES

1 Barnes N D Joseph J M Atherden S M & Clayton B E Functional tests of adrenal axis in children with measurement of plasma cortisol by competitive protein binding *Arch Dis Childh 47* 66 1972
2 Bondy P K & Upton G V Stimultaneous determination of cortisol and corticosterone in human plasma by quantitative paper chromatography *Proc Soc Exp Biol Med 94* 585 1957
3 Brorson I Concentration of corticosterone and cortisol in peripheral plasma of patients with adrenocortical hyperplasia and normal subjects *Acta Endocrinol* (Kbh) *58* 445 1968
4 Bush I E *The chromatography of steroids* Pergamon Press New York–Oxford–London–Paris 1961
5 Buus O Bro Rasmussen F Trolle D & Schacht S Adrenal steroids in mother and infant at birth *Excerpta Med Internat Congr Series 51* 278 196?
6 De Moor P Osinski P Deckx R & Steeno O The specificity of fluorometric corticoid determinations *Clin Chim Acta 7* 475 1962
7 Ely R S Hughes E R & Kelley V C Studies of adrenal corticosteroids Estimation of plasma corticosterone and cortisol *J Clin Endocrinol Metab 18* 190 1958
8 Ferrante L Cortisol Corticosteron und Desoxycortisol Bestimmung im Plasma mit der Transcortin-Cortisol ^{14}C Methode nach Glas Papier Chromatographie (Anwendung bei Fallen von Nebennierenrinden Geschwulsten) *Z Klin Chem Klin Biochem 8* 436 1970
9 Franks R C Diurnal variation of plasma 17 hydroxycorticosteroids in children *J Clin Endocrinol Metab 27* 75 1967
10 Fraser R & James V H T Double isotope assay of aldosterone corticosterone and cortisol in human peripheral plasma *J Endocrinol 40* 59 1968
11 Ganjam V Desjardins C & Ewing L L A quantitative procedure for the determination of

cortisol and corticosterone in blood plasma *Steroids* 16 ??? 1970
1? Hamanaka Y Manabe H Tanaka H Monden Y Uozumi T & Matsumoto K Effects of surgery on plasma levels of cortisol corticosterone and non protein bound-cortisol *Acta Endocrinol* (Kbh) 64 439 1970
13 Hillman D A & Giroud C J P Plasma cortisone and cortisol levels at birth and during the neonatal period *J Clin Endocrinol Metab* ?5 ?43 1965
14 Homoki I & Teller W M Studies on cortisol/corticosterone ratio in plasma as indirect measure of 17 hydroxylase activity during childhood *Acta Endocrinol* (Kbh) Suppl *159* 74 1972
15 Huther K J & Scholz H R Plasma concentrations and rates of plasma clearance and production of *cortisol and corticosterone in healthy persons and in* subjects with asymptomatic and clinical diabetes mellitus *Horm Metab Res* 2 357 1970
16 Hughes E R Seely J R Kelley V C & Ely R S Corticosteroid levels before and after corticotropin *Am J Dis Child 104* 605 196?
17 Iturzaeta N F Hillman D A & Colle E Measurement of pla ma cortisol in children and adults A comparison of the double isotope derivative assay *competitive protein binding analysis and the modified* competitive protein binding analysis *J Clin Endocrinol Metab 30* 185 1970
18 Jubiz W Matsurkura S Meikle A W Harada G West Ch D & Tyler F H Plasma metyrapone adrenocorticotropic hormone cortisol and deoxy cortisol levels *Arch Intern Med 125* 468 1970
19 Martin M M & Martin A L A Simultaneous fluorimetric determination of cortisol and corticosterone in human plasma *J Clin Endocrinol Metab* ?8 137 1968
?0 McLaughlin J Kaniecki T J & Gray I Determination of corticosterone and 17 hydroxy corticosterone in human plasma *Anal Chem 30* 517 *1958*
21 Newsome H H Clements R & Borum E H The simultaneous assay of cortisol corticosterone 11 desoxycortisol and cortisone in human plasma *J Clin Endocrinol Metab 34* 473 197?
22 Painter K Shaw S M & Russell H T A simultaneous assay for cortisol and corticosterone in plasma *Biochem Med* 9 184 1974
23 *Peterson R E & Pierce Ch E The metabolism of* corticosterone in man *J Clin Invest 39* 741 1960
?4 Schweitzer M Branchaud C & Giroud C J P Maternal and umbilical cord plasma concentrations of steroids of the pregn-4-ene ?1-ol sulfate series at term *Steroids 13* 519 1969
25 Stewart C P Alsbert Recht F & O man L M The simultaneous fluorimetric microdetermination of cortisol and orticosterone in plasma *Clin Chim Acta 6* 696 1961
?6 Sweat M C Adrenocorticosteroids in peripheral and adrenal venous blood of man *J Clin Endocrinol Metab* 15 1 043 1955
27 Teller W & Beckmann D Cortisol and corticosterone in human plasma during the newborn period *Acta Endocrinol* (Kbh) Suppl *138* 57 1969
?8 *Van der Vies J Individual determination of cortisol* and corti o terone in a single small sample of peripheral blood *Acta Endocrinol* (Kbh) *38* 399 1961
29 Van der Wal B Israels A L M Janssen J F & Dewied D Some clinical experience in the determination of cortisol and corticosterone in a small sample of human plasma *Acta Endocrinol* (Kbh) *38* 39? 1961

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ANAL TONOMETRY IN THE NEONATAL PERIOD IN MATURE AND PREMATURE CHILDREN

H VERDER P A KRASILNIKOFF and ELMA SCHEIBEL

From the Department of Paediatrics University of Copenhagen Gentofte Hospital Hellerup Denmark

ABSTRACT Verder H Krasilnikoff P A and Scheibel Elma (From the Department of Paediatrics University of Copenhagen Gentofte Hospital 2900 Hellerup Denmark) Anal tonometry in the neonatal period in mature and premature children Acta Paediatr Scand 64 592, 1975 — Anal tonometry was performed during the first days of life in twelve premature and fourteen mature children without signs of gastrointestinal disease A new tonometer with small dimensions was used A pressure decrease of the internal anal sphincter after distension of the rectum could be demonstrated in all children examined and as early as two hours after birth This was to be expected as the relaxation of the internal sphincter is part of the normal defecation reflex Thus anal tonometry can be used in the early diagnosis of Hirschsprung s disease presumable already from the very first days of life

KEY WORDS Anal tonometry newborns Hirschsprung s disease megacolon

A physiological decrease in tone of the internal anal sphincter after distension of the rectum can be demonstrated in older children and adults by means of anal tonometry (4 10 11) This tonus decrease is considered to be part of the normal defecation reflexes (9)

The first passage of meconium in the newborn usually takes place during the first twenty four hours of life Therefore a reflex pattern similar to that of older children can be expected in the newborn though it has not yet been established how early the reflex can be demonstrated

In addition to the physiological importance a clarification of these conditions can be expected to lead to an earlier diagnosis of Hirschsprung s disease In this disease a pressure rise in the internal sphincter is seen in older children after distension of rectum (4 10 11) while the response of the external sphincter shows the same reflex pattern as in normal individuals (10)

The purpose of this investigation has therefore, been to investigate the recto sphincteric reflexes by means of anal tonometry in mature and premature newborn children without gastrointestinal symptoms

As the tonometers used in an earlier investigation (11) were too big for the measurements in the neonatal period a new tonometer with smaller dimensions was constructed

METHODS

The dimensions of the new small tonometer are seen in Fig 1

Balloon A and the tip of the tonometer tube were rubbed with exploration cream Balloon A was placed in the rectum by means of a swab The tube was carefully positioned in the anal canal in such a way that balloon B was just invisible and thus assumed to be in contact with the internal sphincter Balloon C was placed just outside the anal canal Air was insufflated into balloons B and C

Care was taken not to insufflate more than a half ml air into balloon B in order not to dilate the internal sphincter too much and not to disturb the child Balloon A was withdrawn and placed at the top of the tonometer tube

Each balloon was connected by polyethylene tubes with a pressure transducer (Statham Physiological Pressure Transducer P23D6) and the pressure was registered by means of a transducer amplifier (Ellab M5 BCM Copenhagen) together with a special coupling panel for adaption of the pressures on an ultraviolet transscriber (Ultralette abem 5656)

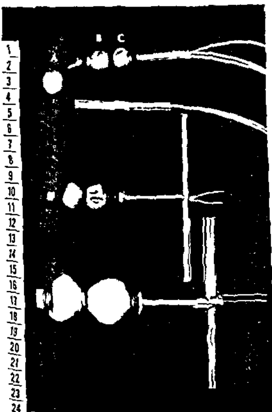


Fig 1 The new small tonometer (with and without balloons) compared with the medium sized (used from 1–4 years of age) and the big tonometer (for older children and adults) The rectal balloon is designated A the internal B and the external C The tonometer tube is made of acryl plast *with an outer diameter of 5 3 mm and an inner* diameter of 4 5 mm

The reflexes were produced by insufflating 1–5 ml air into the rectal balloon

In balloons B and C the pressure was registered continuously Balloon A was only distended at the episodes of air insufflation

Two investigators were necessary one to pacify the child (no premedication was given) and to keep the tonometer in the described position and one to perform the measurements Each investigation lasted from 5– 0 minutes

MATERIAL

Twenty six newborn children (1? premature and 14 mature) without any signs of gastrointestinal disease were examined (Tables 1 and 2) Five children were dysmature 6 demonstrated a picture of tran ient idiopatic respiratory distress syndrome (*IRDS) and 8 were suffering from brief* postnatal asphyxia

RESULTS

The clinical data in prematures appear in Table 1 and in mature children in Table 2

A pressure decrease of the internal sphincter after distension of the rectal balloon was demonstrated in all 26 children examined The decrease *in pressure varied from 2–27 mmHg* and was in some cases initiated by a short positive spike The relaxation of the sphincter varied with *the degree of rectal distension* (Fig 2)

In a few cases a similar pressure pattern but with a smaller amplitude was registered *in the* external balloon as in the internal one This

Table 1 *Clinical data of premature children without gastrointestinal symptoms including age at the first demonstrable relaxation of the internal sphincter*

No	Age (h=hours d=days)	Birth weight (g)	Birth length (cm)	Gestational age (days)	Supplementary clinical diagnosis	Spontaneous activity (contractions/min)
1	4 h	? 50	47	252	IRDS	0
?	1? h	? 450	47	?45	–	0
3	0 h	? 080	46	?46	Asphyxia	0
4	23 h	? ?50	47	6?	Asphyxia	8–9 (46 h)
5	30 h	300	47	250	–	6–7
6	3? h	2 050	45	?	Dysmaturity	8–9 (day 11)
7	47 h	? 00	46	?77	Dysmaturity	8
8	48 h	? 000	46	?6?	Dysmaturity	9
9	4 d	? ?50	45	231	IRDS	7
10	4 d	? 450	47	?82	–	9–10
11	10 d	1 300	40	196	IRDS	0
1? *	0 d	050	46	?36	–	0

Meconium or feces occlusion of the tonometer tube at the first attempt(s) of tonometry
* In child number 1? the relaxation *of the internal* sphincter was registered by an aneroid manometer

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Fig. 1. The new small tonometer (with and without balloons) compared with the medium sized (used from 1–4 years of age) and the big tonometer (for older children and adults). The rectal balloon is designated A, the internal B and the external C. The tonometer tube is made of acrylplast with an outer diameter of 5.3 mm and an inner diameter of 4.5 mm.

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In balloons B and C the pressure was registered continuously. Balloon A was only distended at the episodes of air insufflation.

Two investigators were necessary: one to pacify the child (no premedication was given) and to keep the tonometer in the described position and one to perform the measurements. Each investigation lasted from 5–20 minutes.

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Twenty six newborn children (12 premature and 14 mature) without any signs of gastrointestinal disease were examined (Tables 1 and 2). Five children were dysmature, 6 demonstrated a picture of transient idiopatic respiratory distress syndrome (IRDS) and 8 were suffering from brief postnatal asphyxia.

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2	15 h	450	47	?45	–	0
3	0 h	2 080	46	?46	Asphyxia	0
4	?3 h	? ?50	47	?6?	Asphyxia	8–9 (46 h)
5	30 h	? 300	47	?50	–	6–7
6	35 h	? 050	45	?	Dysmaturity	8–9 (day 11)
7	47 h	? ?00	46	277	Dysmaturity	8
8	48 h	? 000	46	?62	Dysmaturity	9
9	4 d	? 250	45	?31	IRDS	7
10	4 d	450	47	?82	–	9–10
11	10 d	1 300	40	196	IRDS	0
1?*	0 d	2 050	46	?36	–	0

Meconium or feces occlusion of the tonometer tube at the first attempt(s) of tonometry.
* In child number 1? the relaxation of the internal sphincter was registered by an aneroid manometer.

Table 2 *Clinical data of mature children without gastrointestinal symptoms including age at the first demonstrable relaxation of the internal sphincter*

No	Age (h=hours) d=days)	Birth weight (g)	Birth length (cm)	Gestational age (days)	Supplementary clinical diagnosis	Spontaneous activity (contractions/min)
13	2 h	3 500	55	273	–	0
14	10 h	4 000	55	279	Dysmaturity	0
15	12 h	2 750	50	277	Asphyxia	6
16	20 h	3 450	51	257	Asphyxia	0
17	25 h	3 700	52	271	Dysmaturity	0
18	26 h	3 150	51	284	IRDS	0
19	31 h	3 200	50	269	Asphyxia	0
20	32 h	3 150	52	?	Asphyxia	9–10
21	44 h	3 600	55	282	Asphyxia	8
22	3 d	2 600	50	251	–	8
23	3 d	3 200	52	279	IRDS	7
24	3 d	3 850	51	278	Asphyxia	8
25	3 d	4 500	50	271	IRDS	0
26	10 d	3 550	51	?	–	0

Meconium or feces occlusion of the tonometer tube at the first attempt(s) of tonometry

was most likely due to the small anatomic dimensions of the anal canal

In 8 premature and 9 mature children the reflex was demonstrated during the first 48 hours of life The youngest mature child (no 13) examined was 2 hours old and the youngest premature child (no 1) was 4 hours old

The smallest child (no 11) examined had a birth weight of 1 300 g and at the time of investigation (day ten) a weight of 1 290 g

In 2 premature children (nos 6 and 7) a relaxation of the internal sphincter could not be demonstrated at the first tonometric attempt most likely solely due to a meconium plug in the tonometer At the following investigation 24 and 27 hours later respectively the reflex was present In 11 other children (marked with [a] Tables 1 and 2) meconium also plugged up the tonometer tube but after changing the tube from one to three times during the same investigation the sphincteric reflexes could be demonstrated

In 3 patients (nos 13 16 and 19) the reflexes were demonstrable before the first passage of meconium

Spontaneous activity of the internal sphincter with a frequency of six to ten contractions per minute (Fig 3) was found in 13 children (Tables 1 and 2)

In 3 children (nos 4 6 and 8) one to two

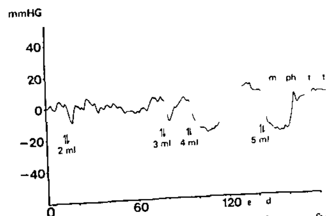


Fig 2 Normal relaxation of the internal sphincter after graduated distension (indicated by arrows) of the rectum in a 26 hour old mature child (Table 2 no 18)

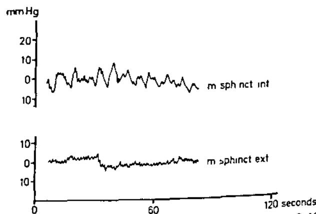


Fig 3 Spontaneous activity of the internal sphincter (9–10 contractions per minute) in a four day old premature child (Table 1 no 10)

-ther tonometries were performed 7 11, d 6 days after the first examination respec -ely No change in the reflex pattern was -served

No complications were observed except for mall anal fissures in 4 cases

DISCUSSION

A relaxation of the internal anal sphincter was found in all mature and premature children examined In nearly all cases the reflex was present during the first investigation even in the youngest child 2 hours old

Free passage of air through the tonometer tube from the atmosphere is a condition of a successful tonometry (10) The tube was often closed by a meconium plug This was the reason why in 11 cases it was necessary to replace the tonometer during the investigation and in 2 cases to repeat the tonometry on the following day

In 17 children relaxation of the internal sphincter was found already during the first 24 hours of life A normal sphincteric reflex has never previously been reported so soon after birth Thus Aaronson & Nixon (1972) (1) and Suzuki et al (1973) (8) could not demonstrate sphincteric reflexes before the third day of life in normal children It also seemed doubtful whether the normal sphincteric reflex pattern was present during the first week of life in prematures and distressed children (1 4)

In the present investigation there was no difference between normal babies prematures and children with IRDS or asphyxia and even in a 4 hour-old premature with IRDS a relaxation of the internal sphincter was found Therefore it is probable that the earlier reports of unsuccessful anal tonometries during the first days of life are due to technical difficulties and not as presumed (4) to immature ganglionic cells or ganglionic cells damaged by anoxia

The present results indicate that the ability of the internal anal sphincter to relax is present at birth This was to be expected as the relaxation of the internal sphincter is part of the normal defecation reflex (9)

Thus the pathological reflex pattern seen in older children with Hirschsprung s disease (10 11) can also be expected to be present at birth

The symptoms of Hirschsprung s disease in the neonatal period can be of a transient nature at first and are therefore often overlooked This compared with an insidious tendency to develop severe enterocolitis makes it important to diagnose Hirschsprung s disease as early after birth as possible Hereby therapeutic intervention (colostomy) can be performed immediately when necessary

Among the various diagnostic facilities available X ray of the colon is often normal during the first months of life (2) A full thickness biopsy of the rectal wall is difficult to perform and may be harmful to the newborn Furthermore the histological picture can be difficult to interpret (5) which in an even higher degree applies to the more superficial suction biopsy of the rectal mucosa (7) On the other hand suction biopsy yields material suitable for determination of the activity of acetylcholinesterase in the mucosa which has been found high in patients with Hirschprung s disease (3 6) Unfortunately the various biochemical methods for the determination of acetylcholinesterase activity are rather complicated and there is no existing normal material for newborns

On the other hand anal tonometry is a simple and rapid method which is not dangerous The present investigation has now made it probable that Hirschsprung s disease can be diagnosed by anal tonometry in the very first days of life in most cases However the technique calls for care in keeping a free passage of air through the tonometer tube limited insufflation of air into the balloons and a gentle handling of the child

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REFERENCES

1 Aaronson I & Nixon H H A clinical evaluation of anorectal pressure studies in the diagnosis of Hirschsprung s disease *Gut 13* 138 1972

2 Ehrenpre s T Megacolon in the newborn A clinical and roentgenological study with special regard to the patogenesis *Acta Chir Scand 94* Suppl 112 1946

3 Elema J D de Vries J A & Vos L J M Intensity and proximal extension of acetylcholinesterase activity in the mucosa of the rectosigmoid in Hirschprung s disease *J Pediatr Surg 8* 361 1973

4 Howard E R & Nixon H H Internal anal sphincter Observations on development and mechanism of inhibitory responses in premature infants and children with Hirschsprung s disease *Arch Dis Child 43* 569 1968

5 Madsen C M *Hirschprung s disease* Munksgaard Copenhagen 1964 p 71

6 Meier Ruge W Lutterbech P M Herzog B Morger R Moser R & Scharli A Acetylcholinesterase activity in suction biopsies of the rectum in the diagnosis of Hirschsprung s disease *J Pediatr Surg 7* 11 1972

7 Noblett H R A rectal suction biopsy tube for use in the diagnosis of Hirschsprung s disease *J Pediatr Surg 4* 406 1969

8 Suzuki H White J J El Shafie M Shaker I J Haller J A and Schnaufer L Nonoperative diagnosis of Hirschsprung s disease in neonates *Pediatrics 51* 183 1973

9 Texter E C Chou C C Laureta H C & Vantrappen G R *Physiology of the gastrointestinal tract* Mosby Saint Louis 1968 chapter 15

10 Tobon F Reid N C R W Talbert J L & Schuster M M Nonsurgical test for the diagnosis of Hirschsprung s disease *N Engl J Med 278* 188 1968

11 Verder H Johansen K S & Engbæk K Anal tonometry A diagnostic help in Hirschsprung s disease *Acta Paediatr Scand 62* 59 1973

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CHRONIC SUPRAVENTRICULAR TACHYCARDIA IN INFANCY AND CHILDHOOD

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ABSTRACT Jacobsen J R Andersen E D Sandøe E Videbæk J and Wennevold A (Medical Department B and Department of Paediatrics Rigshospitalet Queen Louise s Hospital for Children Copenhagen and Departments of Cardiology and Paediatrics Århus Kommunehospital Århus Denmark) Chronic supraventricular tachycardia in infancy and childhood Acta Paediatr Scand 64 597 1975.—The results of a one to 19 year follow up study of 9 children with supraventricular tachycardia of more than one month s duration are reported The ECG diagnosis of tachycardia was made before birth in one patient and between the ages of 8 months and 12 years in 8 Four had sustained and 4 had repetitive tachycardia while one patient had both patterns at different times Reciprocal rhythm was diagnosed in 3 patients and exit block in 2 Severe symptoms had occurred in 2 patients but as a rule symptoms were mild or absent No treatment abolished the arrhythmias but digitalis reduced the overall ventricular rate in 6 patients After a duration of 1–7 years 3 patients still had tachycardia at the follow up In the remaining 6 patients the tachycardia had subsided 7 months to 10 years after the onset

KEY WORDS Repetitive tachycardia sustained tachycardia exit block

Tachycardia is uncommon in infants and children Supraventricular tachycardia is the more frequently encountered (1) while ventricular tachycardia is extremely rare (14) *Paroxysmal* supraventricular tachycardia is the rule but *chronic* supraventricular tachycardia lasting for a longer period of time may also occur The chronic tachycardias may show a repetitive pattern with recurrent bouts of tachycardia beats intervened by sinus beats usually a single or a few in a row or they may take the form of sustained or permanent tachycardias which continue uninterrupted by any sinus node activity (5 10)

In a recently published series including 62 infants and children with supraventricular tachycardia 54 had paroxysmal and 8 chronic supraventricular tachycardia (1) A follow up study on the 54 cases with paroxysmal supraventricular tachycardia was included in the original report The present paper deals with the outcome of a 1 to 19 year follow up study on the 8 infants and children with chronic supraventricular tachycardia plus one other child who was included in the series subsequently

MATERIAL AND METHODS

The series has been collected from two departments of cardiology and three departments of paediatrics from three separate hospitals Case records and lists of diagnoses of two to three decades have been scrutinized and all patients with supraventricular tachycardia of more than one month s duration and with onset before the age of 15 years have been included in the study

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2 Ehrenpreis T Megacolon in the newborn A clinical and roentgenologic al study with special regard to the pathogenesis *Acta Chir Scand* *94* Suppl 112 1946
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4 Howard E R & Nixon H H Internal anal sphincter Observations on development and mechanism of inhibitory responses in premature infants and children with Hirschsprung s disease *Arch Dis Child* *43* 569 1968
5 Madsen C M *Hirschprung s disease* Munksgaard Copenhagen 1964 p 71
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7 Noblett H R A rectal suction biopsy tube for use in the diagnosis of Hirschsprung s disease *J Pediatr Surg* *4* 406 1969
8 Suzuki H White J J El Shafie M Shaker I J Haller J A and Schnaufer L Nonoperative diagnosis of Hirschsprung s disease in neonates *Pediatrics* *51* 183 1973
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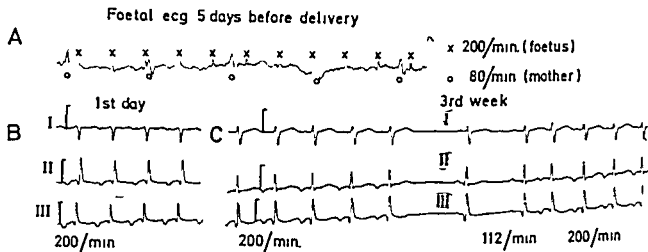


Fig 1 Congenital tachycardia. C P (A) Sustained tachycardia diagnosed from fetal ECG (B) Immediately after birth (C) A repetitive pattern appeared after digitalization PR prolongation before the onset of paroxysms and retrograde P waves suggest a reciprocal rhythm

cardia of more than one month was required for the diagnosis of chronic tachycardia

Recently one patient has been added to the original 8 patients so that the series now includes 9 patients—2 infants and 7 children All patients came for a follow up which included a physical examination ECG and chest roentgenogram The follow up period varied from one to 19 years

RESULTS

Age and sex

In one girl (C P) tachycardia was diagnosed on auscultation four weeks before term A fetal electrocardiogram recorded 5 days before birth showed tachycardia at 200/min similar to ECG tracings achieved after birth (Fig 1)

Besides this case of congenital tachycardia the series includes one infant (L O) in whom tachycardia was diagnosed at an age of 8 months The remaining 7 patients were above 1 year of age at the onset of tachycardia (Table 1) Male/female ratio was 4/5

Predisposing and precipitating factors

Birth weight was above 2 500 g in all cases Onset of tachycardia in one case (M N) was related to a streptococcal throat infection Signs of myocarditis rheumatic fever or chorea were not observed in any case

Additional heart disease thought to be mitral incompetence was seen in one case only (L O) This was a boy who at an age of 8 months was admitted in congestive heart failure A systolic murmur grade 3 was heard at the apex the heart size was grossly enlarged and the ECG showed atrial flutter with a ventricular rate of 200/min The clinical condition of the boy improved by digitalization which reduced ventricular rate but the heart remained somewhat enlarged during the following years The atrial flutter reverted to sinus rhythm at an age of 4 years Last follow up at 8 years of age showed a normally developed boy without symptoms A slight systolic murmur was still present at the apex but heart size was normal sinus rhythm was present and right heart catheterization showed normal pressures

Symptoms and signs

Congestive heart failure which subsided under digitalization was observed in one infant as stated above (L O) At the onset of tachycardia another 3 patients had slight cardiomegaly which gradually subsided A 11 year-old girl (B R) constantly looked pale weak and tired during the morning hours in the first years of her disease and complained later of slight dyspnea and palpitations on exertion One 5 year-old boy (P N) was tired and fainted once or twice during a 6 month period preceding his first hospital admission The

Table 1 Clinical data of nine patients with chronic supraventricular tachycardia

Pat	Sex	Age in years at: First ECG of tachycardia	Last ECG of tachycardia	Follow up	Pattern	Probable Mechanism	Atrial Rate	Treatment	Effects of Treatment
B R	F	11	18	18	Repetitive	Reciprocation	130–220	Digoxin	None
								Alprenolol	None
								Quinidine	None
M F	F	4	5 6/12	23	Repetitive	Reciprocation	154–167	Digitalis	None
M A	F	12	22	?3	Repetitive	Extra systolic	125–180	Digitalis	Reduced pacemaker rate 1 AV block Shorter paroxysms
P C	M	2 6/12	10	10	Repetitive	Extra systolic	125–175	Digoxin	None
								Vagal stimulation	Interruption of single paroxysms
C P	F	Prenatal	1 1/12	4	Sustained/ Repetitive	Reciprocation	160–210	Digoxin	1 AV block Conversion to repetitive tachycardia Abberant ventricular activation
								Diphenylhydantoin	None
M N	M	3 2/12	3 9/12	6	Sustained	Extra systolic	105–?80	Digoxin	None
								Quinidine	None
P N	M	5 11/12	7	7	Sustained	Extra systolic	65–254	Digoxin	2 AV block Exit block
								Digoxin + Propranolol	Exit block
								Practolol	2 AV block
								Quinidine	None
								Propylajmaline	None
								Electroconversion	None
G H	F	1 1/12	6	7	Sustained	Extra systolic	150–220	Digoxin	2 AV block Atrial fibrillation
								Quinidine	None
								Prednisone	None
L O	M	8/12	4	8	Sustained	Flutter	340–380	Digoxin	Increased ? AV block

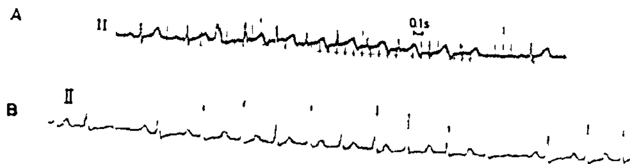


Fig 2 Repetitive tachycardia (A) M I A run of tachycardia is initiated by an atrial premature beat with aberrant QRS Retrograde P waves A reciprocal rhythm is suggested (B) M A Extrasystolic tachycardia suggested from antegrade P waves The PR becomes prolonged during tachycardia and the ectopic pacemaker decelerates towards the end of the paroxysm

immediate cause of admission was a more dramatic event with abdominal pain followed by loss of consciousness cyanosis twitchings and urine incontinence He recovered completely within a few minutes and was fully awake on arrival at the hospital ECG showed atrial tachycardia No abnormality was disclosed by the neurological examination and the electroencephalogram was normal During the following year of treatment he has had no fainting attacks and the only symptoms related to the tachycardia which still continued were occasional periods of weakness and pallor at high heart rates The remaining 6 patients had few and slight symptoms mainly at the onset and in 4 cases the diagnosis of tachycardia was made incidentally during a routine examination by their physician Periods of weakness and pallor lasting from minutes to hours were the most frequently noted symptoms which in 2 patients could be related to an extra rise in heart rate to above 200 per minute One patient (M A) had occasionally experienced slight precordial sensations in adolescence Rapid pulsations on the neck of another child (P C) had worried the parents

ECG at sinus rhythm

Shorter or longer ECG tracings of sinus rhythm were available in all patients Short PQ, WPW pattern or bundle branch block were not observed

ECG during tachycardia

The QRS complexes of tachycardia were narrow and similar to the QRS complexes of sinus rhythm so that ventricular tachycardia might be ruled out

Four patients had repetitive (Fig 2) 4 sustained tachycardia (Fig 3) and one swung between sustained and repetitive tachycardia (Fig 1) The latter patient (C P) was born with sustained tachycardia and changed to repetitive tachycardia during digoxin treatment A change back to sustained tachycardia was observed after withdrawal of digoxin but later on without any therapeutic interventions a conversion to repetitive tachycardia took place The intervening periods of sinus activity consisted of a single or a short row of sinus beats in 4 of the 5 patients in whom repetitive tachycardia was recorded In the fifth case (P C) longer sequences of sinus beats were

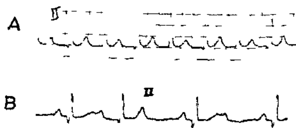


Fig 3 Sustained tachycardia G H (A) Before treatment (B) Second degree AV block with Wenckebach phenomenon appeared after digitalization The reduction in atrial rate was not consistent



Fig 4 Sustained tachycardia with exit block P N (A) At admission rate between 200 (I) and 160 (V_1) per minute A left to right P axis suggests a left atrial pacemaker (B) During treatment with digoxin and propranolol abrupt changes in atrial cycle lengths and a relation between the cycles of 2 3 4 suggest exit block around the pacemaker

the rule but numerous paroxysms were observed daily and the paroxysms often recurred within a few minutes

Mechanism behind the tachycardia

Atrial flutter indicated by the presence of flutter waves with a rate of approximately 350/min was seen in one patient with sustained tachycardia (L O)

Evidence in favour of a circus movement within a longitudinally dissociated AV node a so-named *reciprocating tachycardia* (3 11) was present in 3 cases of repetitive tachycardia The evidence consisted of retrograde P waves (negative P in II III aVF) together with atrioventricular conduction delay (prolonged PR) in the sinus beat or the premature atrial beat which preceded the onset of tachycardia and ventriculoatrial conduction delay (prolonged RP) in the beats of tachycardia (Figs 1 and 2 A)

Tachycardia originating from a focus in the (upper part of) atria so named *extrasystolic atrial tachycardia* (11) was diagnosed in 4 patients 3 with sustained and one with repetitive tachycardia The diagnosis was suggested by the finding of antegrade P waves (+ P in II III AVF) with a rate of 150–280/min and isoelectric shelves in between (Fig 2 B and Fig 3) In one case (P N) the focus might have been located in the upper part of the left atrium as P was negative in I and aVL and dome and dart shaped in V_1 (Fig 4) (11)

Marked variations in atrial rate together with abrupt changes in the P–P interval were observed in 2 cases (P N and M N)

The changes in the P–P interval could be related to a common denominator being a multiple of two three and so on of this denominator (Fig 4) findings which suggest a varying degree of exit block The intermittently observed very high heart rates with a P–P interval equal to the common denominator may have been due to a disappearance of the exit block

In the last patient (P C) ECG tracings of tachycardia did not disclose any signs of atrial activity and consequently no guess could be made about the electrophysiologic background of the tachycardia although no AV conduction disturbances preceded the onset of tachycardia

Treatment

All the patients had been treated with a digitalis preparation and various other antiarrhythmic drugs had been tried in most patients In none did the treatment abolish the arrhythmia neither did electroconversion tried in one patient (P N Table I) with sustained tachycardia However digoxin reduced the ventricular rate in 5 patients either by slowing the ectopic pacemaker or by pro

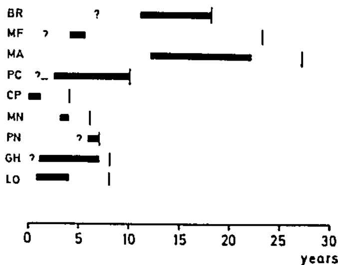


Fig 5 Course of chronic supraventricular tachycardia Solid bars indicate the periods with tachycardia Possible earlier onset according to the history is indicated by a ? Vertical line denotes age at follow up The first 4 patients had repetitive tachycardia and the last 4 sustained tachycardia C P presented both patterns at different times

ducing varying degrees of atrioventricular conduction delay (Fig 3B) In one patient (C P) digoxin changed a sustained tachycardia to a repetitive pattern probably due to inhibition of the A–V conduction as both antegrade and retrograde conduction was prolonged thereby also decreasing the rate slightly The attacks eventually shortened to one or two reciprocal beats Mechanical vagal stimulation could interrupt single paroxysms in a patient with repetitive tachycardia

The exit block around the ectopic pacemaker focus seen in the one case (P N) was only observed during treatment with digoxin either alone or in combination with propranolol, the latter being the more effective The degree of exit block was related to the dose of propranolol Large dosages caused bradycardia and episodes of asystole during sleep which could be prevented by reducing the dose Withdrawal resulted in recurrence of high rate tachycardia In another patient (M N) the presence of exit block could not be related to treatment

Atrial fibrillation lasting from a few days to three weeks was seen repeatedly in one case (G H) during digitalis treatment only

Course and prognosis

All the patients are alive and have developed normally, three of them are now adults (Fig 5)

Three patients (P N P C B R) still had tachycardia at the follow up examination having had tachycardia for 1 7 and 7 years respectively The remaining patients had had no tachycardia for 1 to 17 years at the follow up In these patients the duration of the tachycardia had been 7 months to 10 years (Fig 5 and Table 1) One patient (M A) with extrasystolic repetitive tachycardia still had isolated atrial extrasystoles 5 years after the end of the tachycardia The patient with atrial flutter from his first year of life (L O) got sinus rhythm spontaneously when he was 4 years old A recurrence during right heart catheterization when he was 8 years old was converted to sinus rhythm by d c shock

DISCUSSION

Chronic supraventricular tachycardia is much more rare in infants and children than the paroxysmal type We have found 8 cases among 62 patients with supraventricular tachycardia in our preliminary survey (1) Morgan & Nadas found 10 cases among 53 patients with atrial tachycardia (9) Keane et al recently reported 16 cases seen over 28 years including the 10 cases previously described b Morgan & Nadas (5) A few other series (2 10 13 15 16) and a number of case repor have been published Unlike paroxysm supraventricular tachycardia the chronic ty does not seem to be especially prevalent early infancy (1 12)

In our series of 9 patients additional hea disease was an infrequent finding One patie had auscultatory signs of mitral regurgitatio In another case the onset of tachycardia wa time related to a throat infection but no ev dence of acute myocarditis was present in an of the patients Walters et al (15) stated that of their 5 patients with chronic tachycard had a background of acute myocarditis bu

most other authors on chronic tachycardia in childhood agree that concomitant additional heart disease is infrequent Conversely Levine & Smith (6) found a high incidence of additional heart disease among adults with repetitive tachycardia

The WPW syndrome so frequently observed in infants and children with paroxysmal supraventricular tachycardia (1) was not seen in any of our 9 patients with chronic supraventricular tachycardia and has to our knowledge not been reported in the literature

The paucity of symptoms encountered in children with *chronic* supraventricular tachycardia may be attributed to the moderate degree of tachycardia the heart rate being below 200 per minute in most cases The importance of the heart rate was illustrated in two of our patients who got symptoms when the heart rate on occasions exceeded 200/min Infants with *paroxysmal* supraventricular tachycardia usually have heart rates above 250/min and often develop congestive failure whereas this rarely happens in children who mostly have heart rates below 250 (1 7 12) In one of our 2 infants with *chronic* supraventricular tachycardia the heart rate never exceeded 210/min and she never had any symptoms The other infant who had atrial flutter at a ventricular rate of only 190/min was in severe failure on admission The presence of mitral incompetence and possible myocardial disease may have aggravated his condition

In 6 of our 9 patients the tachycardia had subsided at the follow up having lasted between 7 months and 10 years As early as 1947 Parkinson & Papp writing on repetitive tachycardia stated that children are likely to grow out of it (10) The usual favorable long term outcome has been confirmed in other recent studies (5 16) However the condition may last for many years and patients still having tachycardia after 18 and 22 years of observation have been reported (5 16) The natural course in children with chronic supraventricular tachycardia thus differs from the course in children with paroxysmal supraventricular tachycardia, who rarely get rid of their tendency to recurrences (1, 12)

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REFERENCES

1 Andersen E D Jacobsen J R Sandøe E Videbæk J & Wennevold A Paroxysmal tachycardia in infancy and childhood I Paroxysmal supraventricular tachycardia. *Acta Paediatr Scand* 62 341 1973
2 Dymnicka S Chronic atrial tachycardia in children *Proc Ass Europ Paediatr Cardiol* 8 44 1972
3 Gettes L S & Yoshonis K F Rapidly recurring supraventricular tachycardia A manifestation of reciprocating tachycardia and an indication for propranolol therapy *Circulation* 41 689 1970
4 Hay J D & Keidan S E Persistent ectopic auricular tachycardia in children *Br Heart J* 14 345 1952
5 Keane J F Plauth W H & Nadas A S Chronic ectopic tachycardia of infancy and childhood *Am Heart J* 84 748 1972
6 Levine H D & Smith Jr C Repetitive paroxysmal tachycardia in adults Prognosis–Relationships to nervous system and to classical tachycardia *Cardiology* 55 2 1970
7 Lundberg A Paroxysmal tachycardia in infancy A clinical and experimental study *Acta Paediatr Scand* Suppl 143 1963
8 Mirowski M & Rowe R D Atrial tachycardia with exit block around the ectopic pacemaker in an otherwise healthy infant. *J Paediatr* 80 457 1972
9 Morgan C L & Nadas A S Chronic ectopic tachycardia in infancy and childhood *Am Heart J* 67 617 1964
10 Parkinson J & Papp C Repetitive paroxysmal tachycardia *Br Heart J* 9 241 1947
11 Schamroth L *The disorders of cardiac rhythm* Blackwell Scientific Publications Oxford and Edinburgh 1971 pp 38 44 223
12 Simcha A & Bonham-Carter R E Paroxysmal atrial tachycardia in infants and children *Lancet* I 832 1971
13 Strom G Zetterqvist E & Zetterqvist P Chronic supraventricular tachycardia of continuous or repetitive type in children *Acta Paediatr Scand* 49 827 1960
14 Videbæk J Andersen E D Jacobsen J R Sandøe E & Wennevold A Paroxysmal tachycardia in infancy and childhood II Paroxysmal ventricular tachycardia. *Acta Paediatr Scand* 62 349 1973

15 Walters L Geptes L S Noonan J A & Surawics B (abstract) Long term management of chronic tachycardias of childhood with orally administered propranolol *Am J Cardiol 21* 119 1968
16 Zetterqvist P Long term prognosis of chronic supraventricular tachycardia *Proc Ass Europ Paediatr Cardiol 8* 42 1972

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A STUDY OF SOME BIOLOGICAL AND SOCIO ECONOMIC FACTORS IN LOW BIRTH WEIGHT

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ABSTRACT Bjerre I and Varendh G (Department of Paediatrics Malmo General Hospital Malmo Sweden) A study of some biological and socio economic factors in low birth weight. Acta Paediatr Scand 64 605 1975.—Of 3841 children born alive in Malmo in Sweden in 1966 188 4 9% had a birthweight of ≤2500 g We studied these children with their families and a control group in order to form an opinion on the role played by certain social and biological factors in the aetiology of LBW in a country with a relatively high average standard of living Biological factors were analysed such as the mother s age parity stature and weight as well as purely socio-economic factors such as the mother s civil status the social group of the mother and father income mother s allowance in case of illness social help or investigations regarding one or more members of the family as well as the frequency of immigrants The combined effect of various socio-economic factors was judged by help of a social score The results were analysed for 3 different groups of low birth weight appropriate for gestational age small for gestational age and multiple births Judging from our investigation biological factors probably play the greatest role in the question of LBW but these factors are difficult to distinguish from socio-economic factors which probably still play a certain role in the group preterm children appropriate for gestational age

KEY WORDS Low birth weight gestational age mother s age parity socio-economic factors

The perinatal morbidity and mortality is highest among children with a low birth weight Formerly poor socio economic status was regarded as one of the major causes of low birth weight Baird & Drillien (3 5) for example found a clear correlation between the frequency of low birth weight and low socio economic standard in extensive investigations undertaken in the 1950s in Great Britain Later investigations have not given such clear-cut results (6 14 15 16) It was therefore thought worth while to try to find out whether socio economic factors still play a substantial role in the causation of low birth weight in a population with a generally relatively high standard of living

MATERIAL

The material consisted of all children born alive in the town of Malmö in 1966 and having a birth weight of at most 2500 g In that year Malmö had a population of about 253000 inhabitants Malmo is a one hospital town with one department of obstetrics where all children in the town are born and has a well organized antenatal service which is attended by practically all expectant mothers About 3 2% of all Swedes live in Malmö where the birth rate perinatal mortality and frequency of LBW children is largely the same as that in the country as a whole (8) In 1966 there were 3841 live births in Malmo Of these 188 had a birth weight of at most 2500 g (i e low birth weight = LBW) i e 4 9% These 188 children included 11 pairs of twins and 5 twins whose sibling weighed more than 2500 g at birth 177 mothers and their families were thus examined

The LBW-children were divided into 3 groups viz 1) Children with a short gestational age and weight appropriate for gestational age (AGA) 2) Children with low body weight for gestational age (small for gestational

Table 1 *Social score*

Points	4	3	2	1
Civil status	Married	–	–	Unmarried
Social group				
Father	I	II A	II B	III
Mother	I	II A	II B	III
Income (Sw kr)	≥25 000	15 000–24 999	5 000–14 999	≤4 999
Social help	No	–	–	Yes

age=SGA) and 3) Multiple births The infants were classified as AGA and SGA according to Swedish normal curves for intra uterine growth according to Sterky (12) The word preterm is used to designate children born before the end of the 38th week of pregnancy (≤266 days) The material included 27 twins (43 5% of all twins born in 1966) The twins constituted 15 3% of all LBW infants Of the remaining 161 children 67 (41 6%) were AGA and 94 (58 4%) SGA The control material consisted of those children born alive with a birth weight of more than 2 500 g who were born subsequently to the LBW children according to the register of births The controls for the twins consisted of the next pair of twins in which each child weighed more than 2 500 g

METHODS

Information on the mother s *age parity height weight civil status* at the time of birth of the child and the *occupation* and *nationality* of the parents was extracted from the records of the department of obstetrics The mother s age was noted as the age she attained on her birthday in 1966 irrespective of the month of the year in which the child had been born

The *social classification* was done according to a system devised by Swedner & Gustafsson at the Sociological Institution University of Lund and used in a large number of sociological investigations particularly in south Sweden (13) The classification is based on the occupation and level of education and disregards income Four groups are recognized viz I II*a* II*b* and III *Social group I* comprises owners and managers of large companies senior civil servants and executives in private companies and persons with university degrees *Social group IIa* comprises medium grade civil servants and owners and managers of smaller companies *Social group IIb* consists of lower grade civil servants foremen and tradesmen *Social group III* comprises low grade civil servants skilled and unskilled labourers If the mother has no occupation she is assigned to the same social group as her husband

Information about *incomes* was obtained from the local tax office The incomes used were the gross incomes i e before deduction of income tax and other deductions for the year 1966 and noted in the income returns in 1967 Four income classes were recognised namely at least 25 000 crowns 15 000–24 999 crowns 5 000–14 999 crowns and at most 4 999 crowns If the father s income was known it was his income that was noted—otherwise the mother s income

The mothers annual income for 1966 was not considered representative because she was free from work because of pregnancy for up to 6 months of that year We therefore noted the amount of *sick allowance* she was entitled to in case of illness this sum being a measure of her income before leave of absence because of pregnancy It is also clear from the allowance register whether the mother has any occupation or is a housewife The information was obtained from the 1966 register from the National Health Service

Information about *social support* and social investigations was obtained from the Registers of the Social Welfare Centre in Malmo The help consisted mainly of economic and otherwise supporting social help financial help in case of unemployment and payment for illegitimate children The investigations mostly dealt with alcoholization criminality and who was to be the guardian of the child Each family was regarded as an entity and all help received by one or more of the members was noted The period covered was from the beginning of 1966 to the end of 1970 Social help outside Malmo after a subject had moved from the town was not noted in any of the materials Appointment of guardians was not included because in 1966 such appointments were obligatory for all illegitimate children

Finally the mother s civil status the social groups to which the father and mother belonged income and social help given the family were allotted a certain number of points and the total number of such points was said to be a *social score* (Table 1) We distinguished four groups (5–8 9–12 13–16 17–20 points) where the lowest score denotes the net effect of several negative social factors and means insecurity and poor socio-economic background while the highest score indicates a good social environment and good education

The findings were treated *statistically* with the two-sided Aspin Welch test as well as with a two-sided test using the test quantity

$$t = \frac{p_1 - p_2}{\sqrt{p_T(100 - p_T)\left(\frac{1}{N_1} + \frac{1}{N_2}\right)}}$$

where p_1 is the frequency per cent of a certain factor in a sample of size N_1 p_2 in a sample of size N_2 and p_T in N_1+N_2

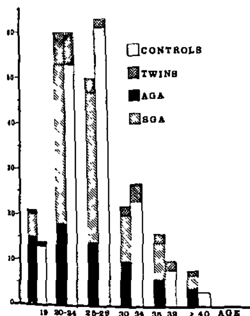


Fig 1 Age distribution of mothers of LBW and control infants.

RESULTS

Mothers age Mothers of LBW children were more common than control mothers in the lowest and in the highest age classes (Fig 1) Of the mothers of LBW children 11 9% (21/177) were at most 19 years old compared with 7 9% (14/177) of the control mothers and 13 6% (24/177) of the LBW mothers were ≥35 years old compared with 7 3% (13/177) of the control mothers These differences were not statistically significant Among the younger mothers there was a preponderance of preterm delivery and 75% (15/20) of the LBW-children of mothers ≤19 years were thus AGA Of the mothers of the AGA children 22 4% (15/67) were ≤19 years old a figure differing significantly from that in the control material (z = 3 2 p<0 01) Among the mothers more than 20 years old no difference of importance was found in the distribution of SGA-children and AGA children The twins were distributed identically among all age groups in the two materials (Fig 1)

Parity Twins were not included in this study as it is known that twin births increase in frequency with the mother s age and parity and because of twins being overrepresented in both materials The percentage of primiparae was higher among the mothers of the LBW children 57 1% (92/161) than among the mothers of control children 46 6% (75/161) (Fig 2) The overrepresentation of primiparae among the mothers of AGA children compared with the control mothers was not large while the difference in primiparity between the mothers of SGA children 56 9% and the mothers of control children 46 6% was statistically significant (z=2 00 p<0 05) Since parity naturally varies with age parity was also studied after exclusion of the ≤19 year age group After such exclusion no difference was found between the mothers of the AGA children and of the controls The difference between the mothers of SGA children 57 3% (51/89) and the mothers of control children 44 6% (66/148) persisted but was not statistically significant (z=1 90 0 05<p<0 10) Only 4–5% of all the mothers had 4 or more children and no difference in this respect was found between the two materials

Mother s stature and weight The average height and weight of the mothers distributed

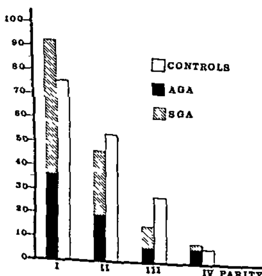


Fig 2 Parity of mothers in LBW and control material Mothers of twins not included

Table 2 *Stature and weight of the mothers in different social groups* (*twin mothers excluded*)

Group	Social groups I+II			Social group III		
		Mean			Mean	
	No	Stature (cm)	Weight (kg)	No	Stature (cm)	Weight (kg)
LBW	67	162 5	53 5	86	162 3	54 7
AGA	24	164 4	54 6	40	162 5	55 6
SGA	43	161 4	52 9	45	162 2	53 9
Controls	75	165 2	56 5	77	163 3	56 6

In the controls the correlation between stature and social group is statistically significant ($p<0$ 05) In LBW no significant differences exist in this respect On the other hand significant differences were found in both stature and weight of mothers between all LBW and controls and between SGA and controls in higher social groups (I and II)

according to birth weight groups and social groups are given in Table 2 The mothers of the LBW children were on the average shorter (162 3 cm) than those in the control material (164 4 cm) This difference did not vary with social class On the contrary the LBW mothers did not show the small differences in stature found between the social groups in the control material The average weight of the mothers in the LBW group was lower than that in the control group Neither was this difference dependent on social group and the average weight relative to height was somewhat larger in the lower social group

Civil status of mother

The distribution of the unmarried mothers in the various groups is given in Table 3 Of the mothers of the LBW children 23 8% (42/177) were unmarried compared with 14 1% (25/177) of the mothers of the control children The difference was statistically significant ($z=2$ 31, $p<0$ 05) This may be regarded as related to age and if mothers ≤19 years old be excluded, the difference 17 3% (27/156) compared with 10 4% (17/163) will no longer be significant The unmarried mothers were most common among the mothers of AGA children where 29 7% were unmarried compared with 20 2% of the mothers of SGA children ($z=2$ 09 $p<0$ 05), this again being related to age

Social group of mother No difference was found between the two materials as a whole or between the different age groups No difference was found between the AGA and the SGA groups

Social group of father The occupation of the father was not known in 16 cases in the LBW group and in 17 cases in the control group No difference in social group was found between the LBW group and the control group but somewhat fewer fathers of AGA children belonged to the highest social groups I and II*a*

Income Income is to be understood as the father s income or if unknown the mother s The median income in the various groups is given in Table 4 The median income in the LBW group was thus lower than in the control group and this was found to be due to a difference between the AGA group and the control group while the SGA group differed only insignificantly from the control group The distribution within the various groups of incomes also showed that a somewhat larger number (12 1%) in the LBW group belonged to the lowest income group (≤4 999 Swedish crowns) than in the control group (8 5%) The differ

Table 3 *Civil status*

	Total	Unmarried	% Unmarried
LBW	177	42	23 8
AGA	67	20	29 7
SGA	94	19	20 2
Controls	177	25	14 7

able 4 *Median income in 1966 counted on le gross income i e before tax deduction* in kr)

,LBW	0 700
GA	19 000
GA	?2 500
ontro s	23 100

nce was due almost entirely to the AGA roup 22 4% of which were in the lowest ncome group The difference between the requency of low income in the AGA group and the control group was statistically signifi cant ($z=2\ 90$ $p<0\ 01$) A low income is natur ally correlated with low age but if the age group below 19 years (mother s age) be ex cluded there will still be a substantial differ ence between the AGA group and the control group in the lowest income group 19 2% compared with 6 8% ($z=2\ 63$ $p<0\ 01$) Ac cordingly in the highest income class (≥25 000 Swedish crowns) the percentage was much higher in the control material 40 2% com pared with 24 1% in the LBW material ($z=3\ 21$ $p<0\ 01$) The difference was found also here particularly between the AGA group (14 9%) and the control group (40 2%) and was statistically significant ($z=3\ 71$ $p<0\ 001$) The SGA group thus differed throughout only slightly from the control group

Sick allowance of mother Owing to emigra tion or death no information was available about 5 cases in the LBW group and in 3 cases in the control group The number of control mothers with the highest sick allow ance 26 4% (47/174) was somewhat larger than that of the LBW mothers 21 5% (37/ 17?) A somewhat larger percentage of the control mothers were housewives 33 3% (58/174) compared with 26 2% (45/172) of the LBW mothers The differences were not statistically significant No difference was found between the mothers of the AGA and the SGA children

Social help and investigations 27 3% of the LBW group and 24 9% of the control group had been in contact with the Social Welfare Authorities In the lowest age group (≤19 years) 62% of the LBW group and only 21% of the control group had been in contact with the Social Welfare Authorities In the other age groups the figure was slightly higher for the control group No difference was found between the families of the AGA and the SGA children The percentage that sought so cial help was high but may be explained by the fact that it refers to a 5 year period to wards the end of which unemployment was relatively high and need of social help natur ally greater

Frequency of immigrants Less than 10% of the mothers of LBW children were immigrants and the same frequency was found among the control mothers

Social score The allotment of points for the various social factors in our social score showed that a low score (5–8 points) was sub stantially more common among the LBW families The difference was statistically significant ($z=2\ 94$ $p<0\ 01$) The difference was found mainly between the AGA families and the control families ($z=3\ 51$ $p<0\ 001$) (Fig 3) while the difference between the SGA families and the control families was not significant ($z=1\ 75$)

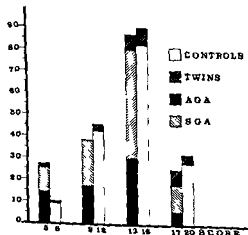


Fig 3 LBW and control material distributed according to presence of various social factors according to a scale covering social group economy civil status need of so cial help Low score=low social standard

DISCUSSION

Socio economic factors are often regarded as a strong contributory cause of low birth weight If this were true improved socio economic conditions should reduce the frequency of LBW children The frequency of LBW infants in Sweden has remained unchanged at about 4–5% (8) during the last decades despite the improved standard of living in all socio economic classes Biological factors such as the mother s age parity height and nutritional as well as gynaecological factors can also contribute to low birth weight and these factors are difficult to separate from purely social variables such as socio economic class economy education and civil status The literature in this field is extensive In the same town as that where our investigation was carried out Malmo Beskow had followed up low birth weight children born in 1929–1939 for 9–17 years (4) He found a clear preponderance of poor social conditions among LBW families From Great Britain several investigations have been published (3 5) Baird & Drillien in Aberdeen and Edinburgh respectively in the 1950s showed a clear correlation between low socio economic class and high frequency of LBW children Drillien showed that the social group to which the maternal grandfather belonged was of greater importance than that to which the father belonged as an expression of the mother s environments and nutrition during childhood She found that social class meant more than income as an expression of the entire socio-cultural background In these investigations it was also found that the woman s height was clearly related to the frequency of LBW children and Baird thought this to be a manifestation of the women in the lowest social classes not having achieved their genetically determined height because of poor nutrition during childhood During the last decade several large investigations have been made in which the socio biological factors have been studied with the aid of multiple factor analysis (6, 9, 16) These investigations have shown that a factor of significant importance when studied alone loses its significance when also other contributory and competing factors are taken into account while the net effect of several negative factors clearly increases the risk of low birth weight

The results of recent investigations are often contradictory probably because of different definition of the parameters used and because of the varying relevance of the same factors in different cultures Our material had the advantage of being representative of the town comprising all LBW children born in that year and of the town of Malmo being fairly representative of a Swedish urban population Like other investigators (1 2 3 9 14) we found that the frequency of LBW varies with the mother s age parity and height

The risk of the child being of low birth weight is greatest if the mother is of early or late fertile age The frequency of premature delivery is highest among young mothers The risk of a low birth weight is also greatest among first borns but this applies to all age groups A short gestational age is thus related to the mother s age while low birth weight for gestational age is more closely related to parity Multiparity is relatively uncommon in Sweden today and could not be judged from this investigation A woman of short stature is more liable to have a low birth weight child but in contrast with what has been found in earlier investigations (3) we found this not to be dependent on the social class to which the woman belonged In Sweden the general standard of living has been relatively good for more than one generation and nutrition of the mothers during childhood has been good which can explain why the social group is no longer of importance for the physical ability of a woman to give birth to a child with normal birth weight

The social class of the mother may also be regarded as a measure of her socio cultural background and education Several investigators have shown that LBW children are more common among mothers belonging to a

r social group with a lower education (3 9 10) We found no effect of these factors ie frequency of LBW-children in our ma l

ie social status of the father is perhaps a er measure of the actual social situation of family than is the mother s We did not find increased frequency of low social group of ers of LBW children Our investigation wed however a greater effect of purely nomic factors The income was lower in LBW group especially in the AGA group le the SGA group did not differ substan y from the control group A lower income s appears to increase the risk of preterm very This difference still exists if the inger mothers are excluded and seems not be dependent only on age

n Sweden there appear to be no ethnic ferences of any significance though the nber of immigrants especially from Fin d and Southern Europe is increasing Most these have a low income but the immigrant thers were not overrepresented in the W material

Of the mothers of LBW children in our ma ial a relatively high percentage were unmar d which is in agreement with what has been and in earlier investigations (1 4 5 9 16) e difference varied partly with age because ost of the unmarried women belonged to the west age classes During later years there s been a change in the family structure in eden so that more and more persons live gether in a stable relationship without being arried But judging from an investigation by owall (11) this tendency had hardly begun 1966 At that time then unmarried women ere as a rule solely responsible for the care f illegitimate children An unmarried mother well accepted in Swedish society but the sponsibility for the child still means an conomic and mental strain which must urely be a social factor of importance It was lso found that the young unmarried mothers f LBW-children needed more help from the ocial welfare than other mothers

The social factors were not predominant in our investigation and they were difficult to judge separately We therefore assigned them a social score and then found that a combination of several negative social factors increases the risk of LBW This applies above all to preterm delivery of children with appropriate weight for gestational age The delivery of children small for gestational age seems due more to other factors such as genetic and obstetric So has for instance the high standard of living resulted in a lower number of children in each family and therefore a relatively large proportion of primiparae who have a higher risk of having children of LBW

The etiology of low birth weight is multifacetted and not to be sought in a single factor but in the combined effect of various early and existing negative social and biological factors which vary from one population to another In several quarters social factors may play a greater role but in Sweden today the biological factors seem to be predominant The effect of negative social factors is still seen to a certain extent mainly among preterm AGA children That biological factors play a greater role for LBW could explain the fact that the total frequency of LBW has not decreased in Sweden despite the improved standard of living

REFERENCES

1 Abramowicz M & Kass E H Pathogenesis and prognosis of prematurity *N Engl J Med 275* 878 1966

2 — Pathogenesis and prognosis of prematurity V *Engl J Med 275* 1001 1966

3 Baird D The epidemiology of prematurity *J Pediatr 65* 909 1964

4 Beskow B Mental disturbances in premature children at school age *Acta Paediatr Scand 37* 125 1947

5 Drillien C M The social and economic factors affecting the incidence of premature birth I Premature births without complications of pregnancy *J Obstet Gynaecol Br Commonw 63* 161 1957

6 Ganguly M Elwood J H & MacKenzie G Factors associated with low and high birth weight *Am J Epidemiol 96* 161 1972

7 Hendricks C H Delivery patterns and reproductive efficiency among groups of differing socioeconomic

status and ethnic origins *Am J Obstet Gynecol 97* 608 1967
8 Public Health in Sweden Annual Report of the National Board of Health 1947–1971 *Official Statistics of Sweden*
9 Rantakallio P Groups at risk in low birth weight infants and perinatal mortality A prospective study of the biological characteristics and socio economic circumstances of mothers in 12 000 deliveries in North Finland 1966 A discriminant function analysis *Acta Paediatr Scand* Suppl *193* 1969
10 Rosenwaike I The influence of socio-economic status on incidence of low birth weight *Health Serv Rep 86* 641 1971
11 Sjövall H Abortion birthrate and marriage in Sweden 1861–1971 *Lakartidningen 70* 3465 1973
12 Sterky G Swedish standard curves for intrauterine growth *Pediatrics 46* 7 1970
13 Swedner H & Gustafsson G Uppdelningen i socialgrupper pp 10–15 *In* H Swedner & G Gustafsson (Compendium March 1964) *Teatervanor och teaterattityder i Malmo åren 1962* University Library Lund Sweden
14 Terris M & Gold E An epidemiologic study of prematurity 1 Relation to smoking heart volume employment and physique 2 Relation to prenatal care birth interval residential history and outcome of previous pregnancies *Am J Obstet Gynecol 103* 358 1969
15 Tulzer W & Wancura I Beinflussung der Fruhgeburtlichkeit durch soziale Faktoren *Arch Kinderheilkd 183* 338 1971
16 Wiener G & Milton T Dermographic correlates of low birth weight *Am J Epidemiol 91* 260 1970

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INCREASED IMMUNOREACTIVE PLASMA AND URINARY GROWTH HORMONE IN GROWTH RETARDATION WITH DEFECTIVE GENERATION OF SOMATOMEDIN A (LARON S SYNDROME)

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ABSTRACT Kastrup K W Andersen H and Hanssen K F (Childrens Hospital Fuglebakken and Steno Memorial Hospital Copenhagen Denmark) Increased immunoreactive plasma and urinary growth hormone in growth retardation with defective somatomedin A generation (Laron s syndrome) Acta Paediatr Scand 64 613 1975.—In a boy 4 years old with clinical hypopituitary dwarfism high plasma and urinary levels of immunoreactive growth hormone were found Somatomedin A levels in serum were low and failed to respond after short term treatment with human growth hormone The parents were first cousins In the arginine and insulin tolerance tests the initially high immunoreactive growth hormone levels were later followed by a decrease to high normal values Insulinopenic response was present during the arginine and glucose tolerance tests As a growth hormone molecule defect is not found in these patients and no growth or other metabolic response to exogenous HGH can be demonstrated it is concluded that a defective somatomedin generation may be present probably in conjunction with a generalized receptor defect and deficient feedback system with abnormal release of HGH The lack of somatomedin A is responsible for the severe growth retardation and the disturbance in carbohydrate metabolism is probably caused by sustained high growth hormone levels

KEY WORDS Growth hormone somatomedin A Laron s syndrome dwarfism

In patients with clinical features resembling hypopituitary growth retardation high plasma levels of immunoreactive growth hormone (IR-HGH) may be found This condition was described originally by Laron (12) as occurring in families of Jewish-oriental extraction

Later Laron et al reported the findings of 26 cases in 14 families in whom the degree of consanguinity was high (16) Furthermore he collected from the literature and by personal communication 15 patients of non Jewish origin from various countries including one case from Sweden In collaboration with Daughaday et al (2) he demonstrated that in patients with this condition a defective generation of somatomedin (SM) was found (previously called sulfation factor)

In the following the clinical features and laboratory studies of a boy with Laron s dwarfism are reported Urinary IR HGH has hitherto not been studied in patients with this syndrome Furthermore the generation of somatomedin is reported after i m and i v HGH Finally immunoreactive insulin levels in plasma in various observations are studied in an attempt to explain the disturbance in carbohydrate metabolism found in these patients

CASE REPORT

B E S a 4 year-old boy firstborn child with birthweight 2 800 g and length 47 cm The parents are both of Danish extraction and are first cousins The pregnancy and delivery were uneventful A younger brother has been examined and is found to be developing normally

In the first years of life he grew slowly Motor development was slow Dentition was late in onset He was

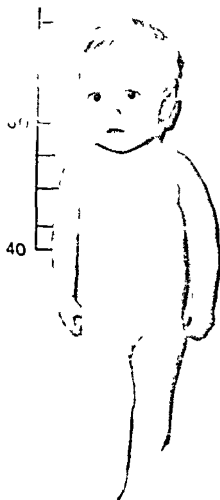


Fig 1 The patient 4 years old

reported to have had from early life episodes of fainting and irritability when hungry He was admitted at the age of 2 years and 3 years because of this and because of his growth retardation which had become more apparent with age

When 2 years old he was admitted to an orthopedic service for several months because of bilateral dysplasia of the hip

On admission at the age of 4 years because of pronounced retardation of skeletal maturation his weight was 9 2 kg height 75 cm (Fig 1) He was obese with small hands and feet and very small external genitalia Testes were not descended Hair growth was sparse frontal bossing with saddle nose and poorly developed mandibula were apparent Teeth were irregular crowded and defective The cranial sutures were not closed The orbits were small and slight proptosis was present The voice was high pitched Mentally he was found to have developed appropriately for his age

Skeletal development was retarded 2 years according to Greulich & Pyle (3) Roentgenograms showed a poorly developed skeletal system with diminished mineralization A normal sella turcica was found

Fig 2 represents the patient s growth chart

Initial growth hormone studies at the Steno Memorial Hospital revealed increased amount of IR–HGH in urine 145 ng/m²/24 h (normal range 20–50 ng/m²/24 h) and high IR–HGH fasting values 75 ng/ml

The patient was then referred to Childrens Hospital Fuglebakken for further studies

Laboratory findings

The hemogram revealed sideropenic anaemia

Plasma creatinine was 55 mmol/l plasma thyroxin on several occasions was found to be normal

METHODS

Plasma and urinary growth hormone was determined by radioimmunoassay according to the method of Hansen (7)

Plasma immunoreactive insulin was determined at Steno Memorial Hospital Research Laboratory with the double antibody method (9)

Determination of somatomedin A (SM) was performed by the method of Hall with modifications (4)

Pelvic leaflets from 11-day-old chicken embryos were incubated for 18 hours in basal Eagle s medium with Hanks salts Serum was added in different concentrations ^{35}S was added to the incubation medium and the incorporation of ^{35}S measured in cartilage with the patient s serum in contrast to a control serum from healthy children

1 unit SM is for practical purposes defined as the biological activity of 1 ml of this serum The assay is based on a symmetrical 4 point design

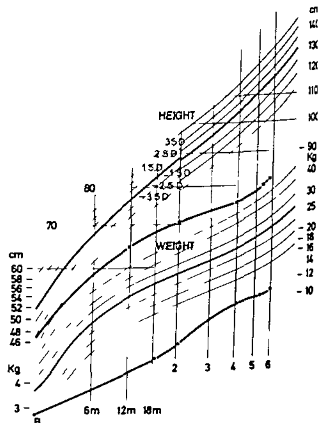


Fig 2 Growth of the patient plotted on a standard growth diagram (Karlberg P et al *Lakartidningen 70* 2963 1973) Part of the diagram is omitted

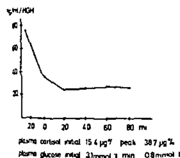


Fig 3 Insulin tolerance test 0 05 IU/kg b w Insulin Leo®

RESULTS

Growth hormone studies

Insulin tolerance test (ITT) (Fig 3) 0 05 IU insulin Leo/kg *in casu* 0 2 units insulin Leo was given i v

The initial value was high and despite an adequate fall in plasma glucose no further rise in HGH could be demonstrated On the contrary a decrease in HGH was noted followed by sustained high basal levels The plasma cortisol response was normal *Arginine stimulation test* (Fig 4) A response identical to that found in the ITT was noted with high initial IR-HGH later followed by a decrease

Somatomedin studies

The SM in the patient s serum was found on several occasions to be low Highest fasting value was 0 37 units SM was studied during the arginine stimulation test after intravenous injection of Nanormon® (human somatotropin) 4 units and after intramuscular injection of the same preparation 8 units daily for 3 days

During the arginine test (Fig 4) no change in SM took place After intravenous injection of HGH (Fig 5) a slight rise in SM was noted

After intramuscular injection of HGH for 3 days fasting SM values were studied for 4 days (Table 1) No significant change in SM levels could be demonstrated

Insulin studies

Plasma immunoreactive insulin was studied during the arginine test (Fig 4) the oral glucose tolerance test and after i v HGH injection An insulinopenic response was found in the first two tests Peak insulin during the arginine test 14 µu/ml during the glucose tolerance test 28 µu/ml (Table 2) Before glucose was given at the end of the HGH injection study there was no change in the plasma glucose or immunoreactive insulin whereas an immediate insulin response was seen afterwards (Fig 5)

DISCUSSION

The patient presented with the symptoms and signs originally described by Laron et al (12 14) and confirmed by personal examination of the patient by Prof Laron The high degree of consanguinity between the parents confirms the autosomal recessive mode of inheritance in this condition

Compared with patients with hypopituitary dwarfism in whom increasing SM values can be demonstrated after treatment with HGH correlated with the growth response and other metabolic responses (nitrogen retention in

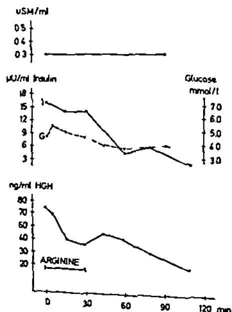


Fig 4 Arginine stimulation test Infusion over 30 min of Arginine 0 5 g/kg b w

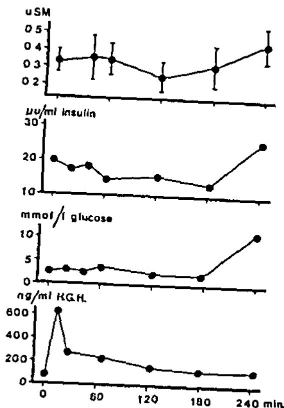


Fig 5 Metabolic response to injection i v of 4 IU HGH 10 ml 50% glucose was given as the patient became drowsy (180 min)

creased free fatty acids and calciuria) such responses cannot be demonstrated in patients with Laron s syndrome either with short term or with long term treatment (2 6 10 15 19 20 21)

When HGH was given intramuscularly for 3 days no change in SM was found This would in hypopituitary patients be an adequate dose to bring low SM values within the normal range according to Daughaday et al (2) As the absence of SM response might be related to the dose of HGH given a single bolus injection was given i v and despite high IR–HGH levels obtained no increase in SM could be demonstrated although this is reported by Hall (5) in hypopituitary subject after 3 hours

As regards the arginine test Laron et al (12), Elders et al (10) and Van den Brande et al (20) point to the inconsistent HGH response with at times even a paradoxical fall in HGH (as demonstrated in our patient) as a characteristic feature As to the ITT, the HGH response has been reported to be higher than normal, unchanged (as in our patient) or in some cases paradoxically low (1 5 10 19 29)

The increased excretion of IR–HGH in urine has not previously been reported Elders et al (10) suggest an abnormal clearance for the HGH as a possible explanation of the findings in this syndrome The increased excretion of IR–HGH in urine in our patient seems to support this, but may also be related to the high plasma levels as in acromegalic patients (8)

It is possible that the high HGH levels at central and peripheral receptor sites is responsible for a refractory state blocking the release and action of HGH This is supported by experimental results cited by Mullers (17) by Elders et al (10) and by Laron et al (15) This would also explain the absence of metabolic responses normally elicited by HGH (10 15 16 19 20)

It is also possible that a feed back system exists in which the HGH release is dependent on the level of SM In conditions with low generation of SM the high HGH production does not overcome the lack of SM most likely due to a defect in SM producing organs at the receptor site (6 10 16)

At the present time the growth hormone molecule in these patients cannot be distinguished from the normal on an immunological isoelectric or biological basis (10, 20) That a growth hormone molecule defect *per se* exists is unlikely as exogenous HGH does not over come the defect The findings therefore suggest that this lack of response to HGH in Laron s dwarfism may be related to a more generalised receptor defect (2 10 19 20)

Table 1 *Somatomedin response to daily injections of human growth hormone 8 IU for 3 days*

	Mean ±S E M
Initially	0 34 U SM ±0 12
Day 1	0 28 U SM ±0 07
Day 2	0 32 U SM ±0 06
Day 3	0 34 U SM ±0 06

Table 2 *Oral glucose tolerance test*

Time (minutes)	0	30	60	90	120	180	240
Glucose mmol/l	3 9	9 0	9 8	8 4	6 8	5 4	4 6
Insulin (μm/ml)	9	6	17	28	15	–	12

Whereas some metabolic responses to HGH are described with no change in SM values a defect of SM formation does not readily explain all the metabolic changes described (4 17) However it seems that in our patient such a defect is the most likely explanation of the findings The disturbance found in carbohydrate metabolism might be induced by the sustained high growth hormone levels

Investigating this disturbance in carbohydrate metabolism Laron *et al* (16) found that a gradual transition from hypoglycemia unresponsiveness to glucose in tolerance characterises these patients Karp et al (11) demonstrated that both parents and patients showed a high incidence of glucose intolerance whereas most of the patients had normal insulin response

The subnormal insulin response after the glucose tolerance and arginine test in our patient has also been reported by others (1 10 11 16 18 20)

Clemons et al (1) reported that long term treatment with HGH was followed by a normalization of the glucose tolerance test with increasing plasma insulin values and suggest a direct effect of HGH on β-cells not mediated by SM as the SM values were not changed In the same study some growth was reported in their patient and it is suggested that an increased insulin production is responsible for this By contrast Van den Brande et al (20) and Najjar et al (18) did not find any change in insulin response on arginine test after long term treatment

The basic defect in Laron s dwarfism has yet to be clarified Further studies of the biological actions of SM are needed

REFERENCES

1 Clemons R Costin G & Kogut M D Laron s dwarfism Growth and immunoreactive insulin following human growth hormone (Abstract) *Pediatr Res 8* 367 1974

2 Daughaday W H Laron Z Pertzelan A & Heins J N Defective sulfation factor generation a possible etiological link in dwarfism *Trans Assoc Am Physicians 82* 129 1969

3 Greulich W W & Pyle S I *Radiographic Atlas of Skeletal Development of the Hand and Wrist* Stanford University Press Stanford Calif 1950

4 Hall K Quantitative determination of the sulphation factor activity in human serum *Acta Endocrinol (Kbh) 63* 338 1970

5 — Effect of intravenous administration of human growth hormone on sulphation factor activity in serum of hypopituitary subjects *Acta Endocrinol (Kbh) 66* 491 1971

6 Hall K & Olin P Sulphation factor activity and growth rate during long term treatment of patients with pituitary dwarfism with human growth hormone *Acta Endocrinol (Kbh) 69* 417 1972

7 Hanssen K F Radio-immunoassay of growth hormone in human plasma *Acta Endocrinol (Kbh) 71* 649 1972

8 Hanssen K F Immunoreactive growth hormone in human urine *Acta Endocrinol (Kbh) 71* 665 1972

9 Jørgensen K R Evaluation of the double antibody radioimmunoassay of insuline and the determination of insulin in plasma and urine in normal subjects *Acta Endocrinol (Kbh) 68* 371 1969

10 Joycelyn Elders M Garland J T Daughaday W H Fischer D A Whitney J E & Hughes E R Laron s dwarfism Studies on the nature of the defect *J Pediatr 83* 253 1973

11 Karp M Laron Z & Pertzelan A Glucose tolerance and insulin secretion in parents and children with familial dwarfism and high plasma immunoreactive growth hormone and familial isolated growth hormone deficiency *Horm Metab Res 4* 284 1972

12 Laron Z Pertzelan A & Mannheimer S Genetic pituitary dwarfism with high serum concentration of growth hormone A new inborn error of metabolism *Israel J Med Sci 2* 152 1966

13 Laron Z Pertzelan A & Karp M Pituitary dwarfism with high serum levels of growth hormone *Israel J Med Sci 4* 883 1968

14 Laron Z Pertzelan A & Frankel J Growth and development in the syndromes of familial isolated absence of HGH or pituitary dwarfism with high serum concentration of an immunoreactive but biologically inactive HGH *In* M Hamburgh & E J W Barrington (eds) *Hormones in development* Appleton-Century-Crofts New York 1971 p 573

15 Laron Z Pertzelan A Karp M Kowadlo-Silbergeld A & Daughaday W H Administration of growth hormone to patients with familial dwarfism with high plasma immunoreactive growth hormone *J Clin Endocrinol Metab 33* 332 1971

16 Laron Z Karp M Pertzelan A Kauli R Keret R & Doron M The syndrome of familial dwarfism and high plasma immunoreactive human growth hormone (IR-HGH) *In* M Pecile & E L Muller (eds) *Growth and growth hormone* Excerpta Medica Amsterdam 1972 p 458
17 Muller E E The nervous control of human growth hormone release In S Raiti (ed) *Advances in human growth hormone research* U S Department of Health Education and Welfare Publication No (NIH) 74-612 1973 p 192
18 Najjar S S Kahchadurian A K Ilbawi M N & Blizzard R M Dwarfism with elevated plasma growth hormone *N Engl J Med 284* 809 1971
19 New J Schwartz E Parks G A Landey S & Wiedemann E Pseudohypopituitary dwarfism with normal plasma growth hormone and low serum sulfation factor *J Pediatr 80* 620 1972
20 Van den Brande J L Du Cajun M V L Visser K A Schopmann W Hackeng W H L & Degenhart H J Primary Somatomedin deficiency *Arch Dis Child 49* 297 1974
21 Van Gemund J J Thesingh C W & Heremans G F P Peripheral subresponsiveness to human growth hormone in the syndrome of familial prenatal nanocephalic dwarfism with high to normal levels of serum immunoreactive growth hormone *Maandschr Kindergeneeskd 40* 369 1972

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CHYMOTRYPTIC ACTIVITY IN STOOL OF LOW BIRTH WEIGHT INFANTS IN THE FIRST WEEK OF LIFE

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ABSTRACT Jodl J Kornfalt R and Svenningsen N W (The Motol Paediatric Clinic University of Prague Prague Czechoslovakia and the Department of Paediatrics University Hospital Lund Sweden) Chymotryptic activity in stool of low birth weight infants in the first week of life Acta Paediatr Scand 64 619 1975.—Pancreatic enzyme activity in low birth weight (LBW) infants during the first postnatal week has been evaluated by analysing the chymotrypsin content of 198 stool specimens from 42 LBW infants with birth weights ranging between 750 and 2 570 g A wide variation in chymotryptic activity yet with a tendency to initially low values with a peak on the third day after birth was found Small for gestational age (SGA) infants had significantly lower values than appropriate-for gestational age (AGA) infants. This is considered due to intra uterine malnutrition with secondary pancreatic dysfunction in SGA infants In screening program for cystic fibrosis or other defects of exocrine pancreatic activity low stool chymotrypsin values cannot be considered pathological until after the fourth day of life

KEY WORDS Chymotrypsin postnatal development low birth weight infants

is well known that the enzyme activities adually appear in the pancreas during the st months of fetal life Chymotryptic and ptic activity has thus been demonstrated in ncreas and meconium in fetuses from the 0-gram stage in the 5th to 6th month of tra uterine development (6) At birth the en matic activities are lower in preterm than in llterm infants although it has been shown by ing test meal (2) as well as by hormonal imulation (10) that preterm babies can se ete substantial amounts of pancreatic en mes These amounts are however consid ably lower than in older infants and children

In studies of the postnatal chymotryptic tivity in stool specimens from healthy full rm neonates Mullinger et al (7) found a de variation during the first days of life yet th a tendency to low values on the first day after birth a peak on the third day and there after falling values Corresponding studies in low birth weight (LBW) infants have not been reported

Thus the aim of the present investigation has been to estimate the chymotryptic activity in stools of LBW infants during the first week of life in relation to 1) postnatal and gestational age 2) birth weight and 3) initiation of peroral feeding

MATERIAL

In 42 LBW infants with birth weights ranging between 750 and 2 570 g stool specimens were collected during the first 7 days of life The total number of specimens analysed was 198 Twenty two infants were appropriate-for gestational age (AGA) and 0 small-for gestational age (SGA) according to internationally accepted definitions (3) During the first 3 days of life 16 infants received parenteral nutrition starting with 5 5% glucose at 2 hours

16 Laron Z Karp M Pertzelan A Kauli R Keret R & Doron M The syndrome of familial dwarfism and high plasma immunoreactive human growth hormone (IR-HGH) *In* M Pecile & E E Muller (eds) *Growth and growth hormone* Excerpta Medica Amsterdam 1972 p 458
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able 1 *Gestational age birth weight and individual values of chymotryptic activity (IU/g stool)* *: 42 LBW infants during the first postnatal week (n s =not significant)*

	Gest age (weeks)	Birth weight (gram)	Postnatal age (days)							Way of nutrition (day 1–3)
			1	2	3	4	5	6	7	
GA	8	1 090	32	–	–	25	–	27	–	Parenteral
	29	1 300	–	62	91	26	–	25	33	Parenteral
	30	1 670	42	63	–	–	34	42	34	Parenteral
	30	1 370	–	–	–	34	–	49	–	Parenteral
	31	1 870	48	52	100	93	30	–	–	Parenteral
	31	1 650	44	–	–	–	–	36	49	Parenteral
	3?	1 500	–	–	36	–	36	62	22	Parenteral
	32	1 790	58	57	80	72	100	125	–	Parenteral
	34	1 710	–	–	49	66	49	–	45	Parenteral
	36	2 100	–	–	35	75	69	35	38	Parenteral
	34	2 380	42	–	–	–	175	–	–	Peroral
	34	2 570	50	–	–	84	–	–	–	Peroral
	34	2 310	30	60	–	–	–	–	–	Peroral
	34	2 270	47	–	–	60	–	–	90	Peroral
	35	2 160	95	48	110	57	65	52	82	Peroral
	35	2 460	46	–	105	–	105	–	–	Peroral
	36	2 230	68	130	–	–	–	–	73	Peroral
	36	2 350	25	68	100	–	–	–	42	Peroral
	36	2 260	?0	55	102	53	48	42	48	Peroral
	36	2 370	–	25	70	77	77	73	80	Peroral
	36	2 4?0	–	80	83	18	42	–	–	Peroral
	37	2 430	15	55	68	46	43	?0	–	Peroral
lean			44 3	62 9	79 1	56 1	67 1	49 0	53 0	
5 E M			5 1	7 1	7 ?	6 3	11 2	8 2	6 4	
5 D			19 2	23 7	24 9	22 7	38 8	27 2	21 4	
V Total number *22*			15	12	13	14	13	12	12	
GA	?9	750	–	0	0	12	–	–	22	Parenteral
	32	750	–	0	0	–	60	150	140	Parenteral
	32	8 0	0	15	–	–	–	130	–	Parenteral
	33	1 200	0	10	45	18	19	52	45	Parenteral
	33	1 400	21	18	39	39	70	80	40	Parenteral
	35	1 350	10	33	50	40	–	–	–	Parenteral
	35	1 770	15	–	90	–	–	–	–	Peroral
	35	1 860	–	0	0	–	60	–	70	Peroral
	36	1 750	55	65	108	1 0	150	100	65	Peroral
	36	1 760	0	40	58	26	36	48	–	Peroral
	36	1 950	–	55	100	60	40	42	75	Peroral
	36	1 510	0	–	11	18	25	62	33	Peroral
	36	2 ?30	55	50	95	68	57	68	90	Peroral
	36	2 30	–	10	90	60	50	70	65	Peroral
	37	2 190	–	15	60	82	45	100	–	Peroral
	37	2 410	0	43	48	43	43	35	80	Peroral
	38	2 440	0	85	80	105	115	105	–	Peroral
	39	1 810	10	–	30	60	75	75	110	Peroral
	40	2 000	40	135	75	155	62	66	50	Peroral
	40	? 3 0	50	65	110	84	78	60	130	Peroral
Mean			18 2	37 5	57 3	61 8	61 5	77 6	72 5	
S E.M			5 8	8 7	8 5	9 9	8 2	7 9	9 4	
S D			21 ?	35 0	24 9	38 5	31 8	30 8	34 0	
V Total number *0*			14	17	19	16	16	16	14	
P value (AGA versus SGA)			<0 002	<0 02	<0 05	n s	n s	<0 02	n s	

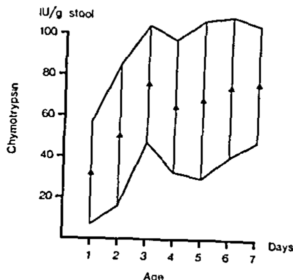


Fig 1 Chymotryptic activity in stool of the 42 LBW infants of the present material during the first postnatal week Mean values ±1 S D

of age and from 12 hours of age a mixture of aminoacids (Vamin Vitrum) fat (20% Intralipid Vitrum) and carbohydrate (5 5% glucose) The parenteral fluids were administered through an umbilical vein (catheter tip in vena cava inferior) or through a scalp vein From the third day of life *peroral feeding through* a nasogastric feeding tube with increasing amounts of human milk was started The other 26 babies were orally fed starting at 6 to 12 hours after birth with human milk or a humanized formula (Milkotal Findus protein content 1 6 g/100 ml) Infants with gastrointestinal disorders were not included in the study

METHODS

The chymotryptic activity was measured according t Haverback (5) using *N* acetyl L Tyrosin-ester (ATEE) a a substrate for the enzyme activity Chymotrypsin in th stool specimen hydrolyses ATEE thereby releasing ace tic acid By titrimetric measurement of the amount of base added to keep pH constant the enzyme activity wa determined using a Radiometer pH Meter 28 with microelectrode chain and Titration Equipment type SBR 2c/ABU/TTT11 (A/S Radiometer Copenhagen) One unit (iu) of chymotrypsin is defined as the activity hydrolysing 1 μmol ATEE substrate per minute at 25°C and pH 7 8 being equivalent to 5 34 μg chymotrypsin According to Haverback values below 74 μg or 13 iu/g stool are pathological while values between 13 and 70 iu/g stool are considered as borderline values Student s *t* test was used for group comparisons

RESULTS

Relation to postnatal age

Fig 1 presents the mean and one standard deviation of the chymotryptic activity of the stools of all LBW infants during the first week of life The average levels are low on the first day after birth but rise to a peak on the third *day of life There is a wide variation* in the chymotryptic activity during the whole first postnatal week

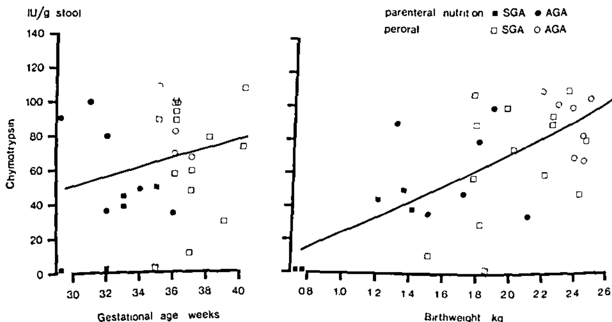


Fig 2 Chymotryptic activity in stool of the 42 LBW infants of the present material on day 3 after birth in relation to gestational age and in relation to birth weight Regression equation and correlation coefficient in relation to birth weight
$y = 43\ 78x - 16\ 46$
$r = 0\ 60$

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	Gest age (weeks)	Birth weight (gram)	Postnatal age (days) 1	2	3	4	5	6	7	Way of nutrition (day 1–3)
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	34	2 [illegible]70	47	–	–	60	–	–	90	Peroral
	35	2 160	95	48	110	57	65	52	82	Peroral
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	36	2 370	–	25	70	77	77	73	80	Peroral
	36	2 4[illegible]0	–	80	83	18	42	–	–	Peroral
	37	[illegible] 430	15	55	68	46	43	[illegible]0	–	Peroral
ean			44 3	62 9	79 1	56 1	67 1	49 0	53 0	
E M			5 1	7 1	7 2	6 3	11 2	8 2	6 4	
D			19 2	23 7	24 9	22 7	38 8	27 2	21 4	
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Total number *20*			14	17	19	16	16	16	14	
value (AGA versus GA)			<0 002	<0 02	<0 05	n s	n s	<0 02	n s	

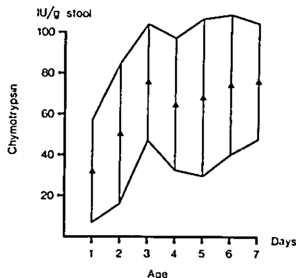


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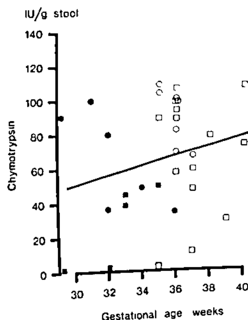
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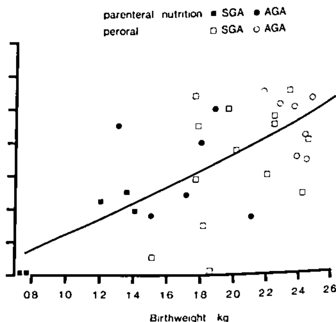


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$y=43\ 78x-16\ 46$
$r=0\ 60$

fants in the first 3 days of life In the SGA infants the intra uterine malnourishment was due to prenatal and fetal infection in 3 babies and placental dysfunction in the other babies In studies of exocrine pancreatic function in children with marasmus and kwashiorkor Barbezat et al (1) have reported very low chymotryptic activity with normalisation within 4 days following protein repletion We propose that the low chymotryptic activity of the SGA infants likewise is a consequence of malnutrition acquired intra uterinely

A dietary influence upon the postnatal development of pancreatic exocrine function has been demonstrated by other investigators (2 10) Our studies have shown a gradual rise of chymotryptic activity in LBW infants during the first days of life In AGA infants no difference was seen between those fed parenterally or perorally However such a difference was obvious in the SGA infants with lower values of chymotryptic activity in the stool of SGA infants initially on parenteral nutrition (vide supra Table 1 and Fig 3) This we consider most possibly to be indicative of a dietary stimulatory effect upon the exocrine pancreatic activity in the first days of extra uterine life As a consequence of our findings we would advocate that in LBW infants with initially low chymotrypsin levels repeated analyses of stool specimens on consecutive days should be performed In screening programs for cystic fibrosis or other defects of exocrine pancreatic activity (8 9) low chymotrypsin values cannot be considered pathological until after the fourth day of life

ACKNOWLEDGEMENTS

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REFERENCES

1 Barbezat G O & Hansen J D L The exocrine pancreas and protein-calorie malnutrition *Pediatrics 42* 77 1968
2 Borgstrom B Lindquist B & Lundh G Enzyme concentration and absorption of protein and glucose in duodenum of premature infants *Am J Dis Child 99* 338 1960
3 Engstrom L & Sterky G Standardkurvor for vikt och langd hos nyfodda barn *Lakartidn 63* 4922 1966
4 *2nd European Congress of Perinatal Medicine* London April 1970 Suggestions concerning the nomenclature for birth weight and gestational age
5 Haverback B J Dyce B J Gutentag P J & Montgomery D W Measurement of trypsin and chymotrypsin in stool *Gastroenterology 44* 588 1963
6 Lieberman J Proteolytic enzyme activity in fetal pancreas and meconium *Gastroenterology 50* 183 1966
7 Mullinger M & Palasi M Tryptic and chymotryptic activity of stools of newborn infants *Pediatrics 38* 658 1966
8 Schmerling D H Prader A Hitzig W H Giedion A Hadorn B & Kuhni M The syndrome of exocrine pancreatic insufficiency neutropenia metaphyseal dysostosis and dwarfism *Helv Paediatr Acta 24* 547 1969
9 Schwachmann H Diamond L K Oski F A & Khan K T The syndrome of pancreatic insufficiency and bone marrow dysfunction *J Pediatr 65* 645 1964
10 Zoppi G Andreotti G Pajno-Ferrara F Njai D M & Gaburro D Exocrine pancreas function in premature and fullterm neonates *Pediatr Res 6* 880 1972

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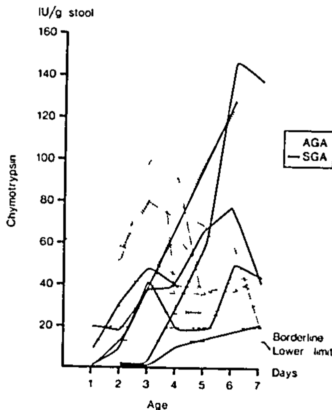


Fig 3 Chymotryptic activity in stool of the 42 LBW infants of the present material receiving parenteral alimentation only during the first 3 days after birth Peroral feeding was initiated in gradually increasing amounts from day 3 after birth Borderline and lower limit according to Haverback (4)

Relation to gestational age and birth weight

Fig 2 shows the chymotryptic activity of samples obtained on the third day of life in relation to gestational age and birth weight respectively Although the wide scatter is conspicuous and no correlation between chymotryptic activity and duration of gestation can be seen there is still a tendency to higher chymotryptic activity with increasing birth weight

The individual values of chymotryptic activity from day 1 to 7 after birth are presented in Table 1 In AGA infants peak values are obtained on the third day of life whereas in SGA infants there is a continuous rise in chymotryptic activity during the first week of life In 12 of 20 SGA but only 1 of 22 AGA infants chymotryptic activity is below 20 iu/g stool in the first specimen obtained (in 9 SGA infants actually 0 iu/g stool) Group comparisons between SGA and AGA infants also show a statistically significant difference in mean values on day 1, 2 and 3 of life the values being lower in the group of SGA infants

Relation to feeding

In SGA infants initially given parenteral nut tion the values of chymotryptic activity a below the mean of the whole SGA group du ing the first 4 days of life (Table 1) The inc vidual postnatal change of chymotryptic acti ity in relation to the first peroral feeding intal in 9 AGA and 6 SGA infants initially c parenteral alimentation is illustrated in Fig : From the third day of life increasing amoun of peroral feeding were given As shown in th figure the chymotryptic values are conspict ously lower in SGA infants than in AGA ir fants during the initial period of parenter: nutrition However the number of analyse stool specimens in infants parenterally fed i too small to allow of any statistical evaluatio: of group differences between AGA and SG/ infants in this respect

DISCUSSION

In a study of the exocrine pancreas functioi response to pancreozymine and secretin Zoppi et al (10) showed that at 2–6 hours afte birth the pancreatic enzyme activities (α amylase trypsin and lipase) in duodenal juice were lower in preterm than in fullterm infants At 24 hours of age there was no significant difference and at the end of the first week equal or even higher exocrine pancreatic activ ity was found in preterm babies The findings of the present investigation showing that LBW infants generally have low chymotryptic activity in stool specimens during the first days of life followed by increasing values towards the end of the first postnatal week are in agreement with the abovementioned observations

In addition the present investigation has shown that the chymotryptic activity is significantly lower in SGA than in AGA in

Table 1 *Immunologically measured antithrombin III concentration in different age groups*

Age group	Number of patients	% of normal adult Mean±S D	Range
I 4–7 days	36	45 2±12 0	26–68
II 1–4 weeks	36	54 2±13 0	20–79
III 1 12 months	46	82 8±18 7	53–122
IV 1 14 years	51	105 6±23 3	55–166

Table 2 *Antithrombin activity in different age groups*

Age group	Number of patients	% of normal adult Mean±S D	Range
I 4–7 days	25	65 0±22 1	11–104
II 1–4 weeks	27	71 7±15 6	48 106
III 1–12 months	29	89 6±16 5	63–1.0
IV 1–14 years	33	97 9±14 5	73–136

MATERIAL AND METHODS

Clinical material

1 34 normal newborn infants 4–7 days of age treated at a maternal care department The blood was taken in connection with routine blood sampling for phenylalanine determinations

2 149 patients aged 1 week to 14 years admitted to a pediatric clinic under various mainly surgical diagnoses but without evidence of bleeding tendency and diseases influencing the coagulation system The blood was taken in connection with preoperative screening analysis

3 30 unselected hospitalized infants 0–6 months of age with various diseases Antithrombin was measured in connection with routine thrombotest analyses

Plasma from patients in groups 1 and 2 was used for establishing reference values Due to shortage of plasma both methods were not performed in all individuals Plasma from patients in group 3 was used for correlation between antithrombin and thrombotest

Collection of blood

Capillary blood 180 µl was taken from a heel or a finger prick into a 00 µl microcap containing 20 µl of 0 13 M trisodium citrate solution The blood was transferred to plastic microtubes plasma was separated (Microfuge 152 Beckman Spinco Geneva) and stored frozen

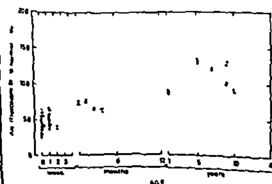


Fig 1 Immunologically measured antithrombin III in infants and children 0–14 years of age

Determination of antithrombin in plasma

1 Single radial immunodiffusion technique according to Mancini (21) as described by Abildgaard (10) Antiserum against antithrombin III (Nygaard & Co Oslo) was used

2 A micromodification of the method described by Svendsen Blomback and co-workers (26 6) In this procedure thrombin is incubated in heat-defibrinogenated plasma and the residual thrombin activity determined by aid of a synthetic tripeptide substrate for serine proteases (Bofors Nobel Division Molndal Sweden)

For both methods antithrombin was evaluated from a standard curve obtained by diluting a pooled reference plasma from twenty healthy young males Antithrombin was expressed in per cent of this plasma

Thrombotest was measured in capillary blood and the values were corrected to a hematocrit of 45%

RESULTS

Antithrombin in different ages

The antithrombin III concentration as found by the immunological method are set out in Table 1 and Fig 1 In the first month of life antithrombin III concentration was only about half of the adult value It increased with age and at about 6 months was roughly the same as in adults In early childhood it seemed to be even slightly higher than in adults When the clinical material was divided into age groups as indicated in Table 1 the antithrombin III concentrations of all age groups differed significantly from one another ($p<0.001$)

The antithrombin activity is shown in Table 2 and Fig 2 With this method too antithrombin levels were considerably lower in young infants than in adults and increased with age to reach adult values at about half a year of age The antithrombin activities in the two lowest age groups did not differ significantly from each other Otherwise antithrombin

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ANTITHROMBIN IN INFANCY AND CHILDHOOD

ANN-CATRINE TEGER NILSSON

From the Department of Clinical Chemistry Department of Pediatrics and Department of Blood Coagulation Research Karolinska sjukhuset Stockholm Sweden

ABSTRACT Teger Nilsson A C (Department of Clinical Chemistry Department of Pediatrics and Department of Blood Coagulation Research Karolinska sjukhuset Stockholm) Antithrombin in infancy and childhood Acta Pediatr Scand, 64 624 1975.—Antithrombin III was measured immunologically, and antithrombin activity was measured with aid of a new synthetic tripeptide substrate for serine proteases in plasma of healthy infants and children, 0–14 years of age Both methods gave decreased values in the youngest infants as compared with adults The antithrombin increased with age and reached adult values about 6 months of age In infants up to one month of age antithrombin III measured immunologically was significantly lower than antithrombin activity, whereas there was no difference between the antithrombin methods in the higher ages Antithrombin III concentration and antithrombin activity as measured were poorly correlated

KEY WORDS Antithrombin, infants children

The coagulation and fibrinolytic systems in young individuals especially newborns show several differences from those of adults The naturally occurring antiproteases in plasma likewise differ the most outstanding finding being a high α_2 macroglobulin concentration in childhood (12 9) The role of different antithrombins in haemostasis is of increasing interest, as reliable and easily performed methods are developed Antithrombin III is probably the most important naturally occurring antithrombin (1 2) It is shown to be decreased in patients with impaired liver function (7, 14, 13) in women taking oral contraceptives (10, 15), in pregnancy post partum and postoperatively (11, 16, 18) and to a certain extent in intravascular coagulation (19 14) Low antithrombin III values have also been found in some families with high incidence of thrombosis (8, 14 22, 24)

Antithrombins in childhood are less studied Several authors however, have noticed decreased antithrombin activity in cord blood Stroder & Kunzer found an increased antithrombin II activity and decreased antithrombin III activity in premature newborn (25) Krause & Maus using an immunological method reported on decreased antithrombin in newborns (17) whereas Bergin et al using activity methods found values mainly within the range for adults (3) Low values with an activity method were noted by Biland & Duckert (4) and Mahasandana & Hathaway (20) The latter authors in addition found particularly low values in infants developing IRDS A correlation between asphyxia and low antithrombin was also recently described by Weissbach et al (27)

In the present paper antithrombin has been measured in infants and children both immunologically with antibody against antithrombin III and as antithrombin activity with aid of a new substrate for serine proteases

Table 1 *Immunologically measured antithrombin III concentration in different age groups*

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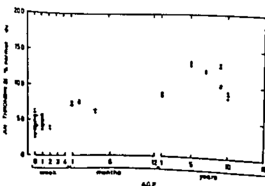


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The coagulation and fibrinolytic systems in young individuals, especially newborns show several differences from those of adults The naturally occurring antiproteases in plasma likewise differ the most outstanding finding being a high α_2 macroglobulin concentration in childhood (12, 9) The role of different antithrombins in haemostasis is of increasing interest as reliable and easily performed methods are developed Antithrombin III is probably the most important naturally occurring antithrombin (1 2) It is shown to be decreased in patients with impaired liver function (7, 14, 13), in women taking oral contraceptives (10 15), in pregnancy post partum and postoperatively (11, 16, 18) and to a certain extent in intravascular coagulation (19 14) Low antithrombin III values have also been found in some families with high incidence of thrombosis (8, 14, 22 24)

Antithrombins in childhood are less studied Several authors, however, have noticed decreased antithrombin activity in cord bloo[d] Stroder & Kunzer found an increased ant[i]thrombin II activity and decreased ant[i]thrombin III activity in premature newborn[s] (25) Krause & Maus using an immunologic[al] method reported on decreased antithromb[in] in newborns (17) whereas Bergin et al using activity methods found values mainly within the range for adults (3) Low values with a[n] activity method were noted by Biland & Duckert (4) and Mahasandana & Hathaway (20) The latter authors in addition found particularly low values in infants developing IRDS A correlation between asphyxia and low antithrombin was also recently described by Weissbach et al (27)

In the present paper antithrombin has been measured in infants and children both immunologically with antibody against antithrombin III and as antithrombin activity with aid of a new substrate for serine proteases

exerts thrombin activity on the tripeptide substrate (6) Occurrence of a fetal antithrombin III with other immunological properties may also be taken into consideration The poor correlation between the two antithrombin m thods also indicates that they measured different and probably uncorrelated parameters

Antithrombin III concentration and antithrombin activity reached adult values at the age at which prothrombin and factors VII X and IX are also known to reach adult values (23) There was also a correlation although slight between antithrombin and thrombotest in infants below 6 months of age The findings indicate a coupling of the synthesis of prothrombin and antithrombin i e the precursor of a protease and its antiprotease in the developing child Other reasons for the correlation such as liver disease and increased consumption of both prothrombin-like factors and antithrombin cannot however be excluded considering the unselected material used in this study

Both methods require only small amounts of plasma and can be performed in capillary blood They are therefore convenient for infants The clinical value of antithrombin III measurements in infants and children is still not known As in adults it may contribute to the diagnosis of disseminated intravascular coagulation and liver diseases Recently there appeared a report on two women with inherited antithrombin deficiency who delivered infants with a high incidence of deadly thrombosis (5) Antithrombin should therefore be measured in newborns with antithrombin III deficiency in the family and perhaps in all situations with unexpected thrombosis in infancy and childhood

ACKNOWLEDGEMENT

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REFERENCES

1 Abildgaard U Inhibition of the thrombin fibrinogen reaction by antithrombin III studied by N terminal analysis *Scand J Cl n Lab Invest 20* 207 1967

2 — Antithrombin III en viktig fysiologisk koagulationshemmer *Farmakoterapi 27* 2 1971

3 Bergin J J Zuck T F Corby D G & Shigeta F H Effect of childbirth on maternal and fetal antithrombin III activity Fourth International Congress on Thrombosis and Haemostasis Vienna 1973

4 Biland L & Duckert F Coagulation factors of the newborn and his mother *Thromb Diath Haemorrh* 29 644 1973

5 Bjarke B Herin P & Blomback M Neonatal aortic thrombosis A possible clinical manifestation of congenital antithrombin III deficiency *Acta Pediatr Scand 63* 297 1974

6 Blomback, M Blomback B Svendsen L & Olsson P The assay of antithrombin using a synthetic chromogenic substrate for thrombin *Thrombosis Res* Submitted for publication

7 Duckert F Behaviour of antithrombin III in liver disease *Scand J Gastroenterol* Suppl *19* 109 1973

8 Egeberg O Inherited antithrombin deficiency causing thrombophilia *Thromb Diath Haemorrh 13* 516 1965

9 Ekelund H Hedner U & Nilsson I M Fibrinolysis in newborns *Acta Paediatr Scand 59* 33 1970

10 Fagerhol M K & Abildgaard U Immunological studies on human antithrombin III Influence of age sex and use of oral contraceptives on serum concentration *Scand J Haematol 7* 10 1970

11 Fischer M Verhalten menschlicher Thrombininhibitoren (α-Makroglobulin und Antithrombin III) während und nach chirurgischen und gynäkologischgeburtshilflichen Eingriffen *Folia Haematol* (Leipz) *98* 397 1972

12 Ganroth P O & Schersten B Serum α-macroglobulin concentration and its variation with age and sex *Clin Chim Acta 15* 113 1967

13 Hallén A & Nilsson I M Coagulation studies in liver disease *Thromb Diath Haemorrh 11* 51 1964

14 v Kaulla E & v Kaulla K N Antithrombin III and disease *Am J Clin Pathol 48* 69 1967

15 v Kaulla E & v Kaulla, K N Oral contraceptives and low antithrombin III activity *Lancet I* 36 1970

16 v Kaulla E Droegemeuller W Aoki N & v Kaulla K N Effect of oestrogens on postpartum hypercoagulability and antithrombin III activity *Am J Obstet Gynecol 113* 9 0 1972

17 Krause W & Maus W Die Bedeutung der Reptilasezeit zur Frage des fetalen Fibrinogens Klin Wochenschr 51 94 1973

18 Kunz S Herschlein H J Steichele D F & Kidess E Verhalten physiologischer Inhibitoren der Blutgerinnung und Fibrinolyse während der Schwangerschaft, unter der Geburt und im Wochenbett Immunologische Untersuchungen *Med Welt 24* 1 66 1973

19 Lasch H G Rodriquez Erdmann F & Schimpf K Antithrombin III und Anti Blutthrombokinase bei experimenteller Verbrauchkoagulopathie *Klin Wochenschr* 39 645 1961

20 Mahasandana C & Hathaway E W Circulating anticoagulants in the newborn Relation to hyper

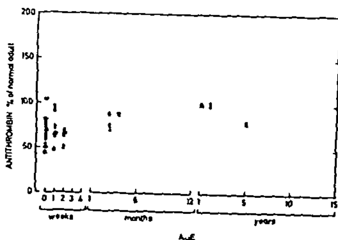


Fig 2 Antithrombin activity in infants and children 0–14 years of age

activities were different in the different age groups ($p<0.001$–<0.05)

Infants up to one month of age showed significantly lower values for antithrombin III measured immunologically than for antithrombin activity ($p<0.001$) whereas there was no difference between the two methods in the older ages

Correlation between antithrombin methods

In altogether 89 patients representing all involved ages antithrombin was analysed with both methods in the same plasma sample There was a poor correlation between the methods (correlation coefficient 0 1–0 4) except for the newborns 4–7 days old in whom the two antithrombin methods correlated somewhat better (correlation coefficient 0 7)

Correlation between antithrombin and thrombotest

An attempt was made to see if there was a correlation between antithrombin and prothrombin like factors i e between an antiprotease and its protease or protease precursors Prothrombin like factors were estimated by thrombotest, in unselected hospitalized sick infants The results, shown in Fig 3, indicate a slight correlation between antithrombin and thrombotest, with a correlation coefficient of 0 6 for both methods of antithrombin determination

DISCUSSION

Antithrombin III measured immunologically was clearly depressed in newborns as was antithrombin activity, estimated by its ability to inactivate thrombin This is in agreement with earlier findings (3 4 17 20 25) The levels in the youngest infants were of the same magnitude as in adults with inherited antithrombin III deficiency and increased thrombosis tendency (8, 14, 22 14) Thrombosis is however, a minor problem in newborns It therefore seems probable that the low values of prothrombin and prothrombin like factors in infants counteract the coagulation promoting activity of a low antithrombin value Other differences in the haemostasis mechanism may also contribute to obtain a balanced haemostatic situation in infants

The antithrombin activity showed somewhat higher values than immunologically measured antithrombin III in the youngest infants This finding implies an influence from other antiproteases α_2 Macroglobulin is known to be high in infancy and childhood (12 9) but the influence of this antiproteases is difficult to evaluate as it is shown that the complex of thrombin and $\alpha_2$macroglobulin still

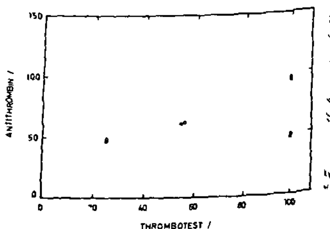


Fig 3 Correlation between thrombotest and antithrombin in infants 0–6 months of age ● immunologically measured antithrombin III ○ antithrombin activity

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NEUROLOGICAL FINDINGS AT FOLLOW UP IN NEONATAL HYPOGLYCAEMIA

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ABSTRACT Fluge G (Department of Paediatrics University of Bergen Bergen Norway) Neurological findings at follow-up in neonatal hypoglycaemia Acta Paediatr Scand 64 629 1975.—Follow-up examination was carried out in 37 children who had been hypoglycaemic during the neonatal period Mean age was 3 1/2 years Five out of 7 children with asymptomatic hypoglycaemia neonatally were completely normal while minimal brain dysfunction was evident in one and another child showed pathological EEG Symptomatic transient hypoglycaemia seemed to carry a poor prognosis as only one out of 9 individuals was normal Four patients in this group had convulsions after the neonatal period two of these had recurrence of hypoglycaemia One had infantile spasms and was severely mentally retarded with spastic diplegia and epilepsy One girl was blind due to optic nerve atrophy Four cases of cerebral palsy were detected in this group Among 21 cases of secondary hypoglycaemia there were no cases of serious neurological sequelae It is reasonable to assume that neonatal hypoglycaemia is an important prognostic factor The deleterious effect on the CNS seems to be related to the duration and severity of the hypoglycaemia

KEY WORDS Neonatal hypoglycaemia follow-up studies growth and development

Permanent central nervous system (CNS) damage following neonatal hypoglycaemia has been reported by several authors (1 2 3 4 5 1 12 13 14) However opinions differ as to whether the hypoglycaemia *per se* caused brain damage in these patients or whether it is only a secondary phenomenon in neonatal disorders such as asphyxia brain injury at birth or respiratory distress This controversy emphasizes that the extent of CNS damage due solely or mainly to hypoglycaemia is difficult to assess or to separate from other concurrent cerebral birth injuries

The present paper reports the neurodevelopmental status at follow up of different categories of neonatal hypoglycaemia with special reference to the prognostic significance of the duration of hypoglycaemia and occurrence of convulsions in the neonatal period

MATERIAL AND METHODS

During the 3 year period 1967–69 hypoglycaemia was encountered in 67 newborn infants at the Department of Paediatrics University of Bergen Fifty cases were detected by routine blood glucose determinations among 323 low birth weight infants and the remaining 17 patients were full term newborns The infants were classified in three groups according to clinical assessment Thirteen patients (*Group A*) had *asymptomatic* hypoglycaemia Eleven cases (*Group B*) were classified as *symptomatic transient* hypoglycaemia defined as symptomatic hypoglycaemia responding to glucose infusions and with no other apparent neonatal disorder Hypoglycaemia concomitant with neonatal complications such as asphyxia brain injury and respiratory distress and with unsatisfactory response to glucose infusions was classified as *secondary* hypoglycaemia (*Group C* 43 cases) Groups B and C were further divided into a convulsion and non convulsion group Details with regard to the clinical course during the neonatal period have been published elsewhere (6) Nineteen patients died neonatally and one patient died later in infancy Nine cases were lost at follow-up and one patient was excluded because of Down s syndrome Thus 37 cases have been followed up The mean age was 3 1/2 years (range [illegible] years)

A questionnaire on the development of the infant was

coagulability and the idiopathic respiratory distress syndrome *Pediatr Res 7* 670 1973
21 Mancini G Carbonara A O & Heremans J F Immunochemical quantitation of antigens by single radial immunodiffusion *Int J Immunochem 2* 235 1965
22 van der Meer J Stoepman van Dalen E A & Jansen J M S Antithrombin III deficiency in a Dutch family J Clin Pathol 26 532 1973
23 Noren I Carlsson E Kretzschmar G & Teger Nilsson A-C Prothrombin in newborns and during the first year of life *Acta Pediatr Scand 60* 269 1971
24 Penick G D Blood states that predispose to thrombosis *In* S Sherry K M Brinkhaus E Genton & J M Stengle (eds) *Thrombosis* National Acad Sci Washington D C 1969 p 553
25 Stroder J & Kunzer W Gerinnungsstudien bei Kindern XIII Mitteilung Antithrombin II und Antithrombin III bei Unreifgeborenen *Ann Paediatr 196* 342 1961
26 Svendsen L Blomback B Blomback M & Olsson P Synthetic chromogenetic substrates for determination of trypsin thrombin and thrombin like enzymes *Thrombosis Res 1* 267 1972
27 Weissbach G Domula M Lenk H & Schneider P The progressive antithrombin activity and its relations to other factors of the coagulation system in newborns *Acta Paediatr Scand 63* 555 1974

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Table 2 *Data on 5 patients with neonatal hypoglycaemia and cerebral palsy at follow up*

Case	Category of neonatal hypoglycaemia	Birth weight (grams)	Gest age (weeks)	Complications during delivery	Condition at birth	Lowest blood glucose level (mg/100 ml)	Duration of hypoglycaemia
O E male	Symptomatic transient	2 300	38	–	Fair	7	>12 hrs
R. D male	Symptomatic transient	3 030	41	Possible foetal distress	Good	3	>12 hrs
T S female	Symptomatic transient	1 700	33	–	Fair	10	<3 hrs
J B male	Symptomatic transient	1 470	36	–	Good	9	>12 hrs
L N female	Secondary	1 870	32	Placenta praevia malposition forceps delivery	Severe asphyxia	8	<4 hrs

a complicated delivery and two blood glucose readings before the age of 4 hours were 16 and 8 mg/100 ml Except for mild ataxia however no other neurological or intellectual handicaps could be demonstrated in this child

Developmental assessment disclosed abnormal findings in 11 cases (30%) as shown Table 3 One group A patient had minor otor and behaviour disturbances indicating BD He had initial blood glucose readings of and 6 mg/100 ml without any symptoms and continued to show a tendency to hypo lycaemia during the first 5 days of life

Four out of 9 children in group B showed ormal mental development although 3 of hem had hypoglycaemia of more than 12 ours duration and 2 were convulsive However cerebral palsy was documented in 2 of hese patients Two children had signs of MBD one was severely mentally retarded and 2 were doubtful Prolonged hypoglycaemia had been present in all group B patients with developmental handicaps and all but one of them had had neonatal convulsions

In group C 16 out of 21 children were mentally normal 4 of them had convulsions during the neonatal period but hypoglycaemia was of short duration and did not coincide with the convulsions in these individuals Among the remaining 5 patients from this group one was grossly retarded He had been asphyxiated at birth and initially respiratory distress was present Blood glucose recordings of 19 and 15 mg/100 ml were noted before the age of 6 hours and the subsequent neonatal course was unremarkable Two other patients showed delayed speech development one of them was found to be moderately mentally retarded on formal intelligence testing One patient in this group had minor motor disturbances suggesting MBD and one was questionable in this respect Among group C patients who showed developmental defects there was only one who had had hypoglycaemia of more than 12 hours duration and none of them were convulsive neonatally

Assessment of *somatic growth* disclosed no cases of short stature The height was below the 25th percentile in 8 children while it exceeded the 75th percentile in only three Correspondingly the body weight according to height was below the 25th percentile in 17 children and only 3 individuals had body weight above the 75th percentile The head circumference was above the 97 5 percentile in 4 cases Apart from this they were normal and there were no other findings suggesting a hydrocephalus None of the children had a head circumference below the 2 5 percentile

Table 1 *Findings at follow up in neonatal hypoglycaemia*

Category of neonatal hypoglycaemia	No of cases	Normal findings	Severe mental retardation	Minor developmental defects	Cerebral palsy	Abnormal EEG findings	Squint	Later convulsions	Hypoglycaemic episod
Asymptomatic	7	5	–	1	–	2	–	–	–
Symptomatic transient									
Convulsion group	6	1	1	1	2	4	2	3	2
Non-conv group	3	–	–	1	2	1	1	1	–
Secondary									
Convulsion group	4	1	–	–	1	1	–	–	–
Non conv group	17	9	–	4	–	2	1	–	–

sent to the parents several months before the examination and further details were obtained during an interview In addition to a complete neurologic and somatic status the developmental level concerning gross motor fine motor language and personal social behaviour was assessed Screening for hearing defects by audiograms was carried out at the Institute for Hearing Defects in Bergen and if indicated repeated after a year A complete eye examination including investigation of the lens by slit lamp to detect zonular cataracts was made by an ophthalmologist An electroencephalogram (EEG) was obtained on at least one occasion

The children were considered normal if their development was within normal limits and there were no signs of neurological damage including normal findings by audivisual and electroencephalographic examination

RESULTS

Of 37 children examined 16 (43%) were completely normal while in the remaining 21 (57%) one or more pathological findings were noted These findings are presented in Table 1 It appears that the most severe sequelae were detected in group B namely those 6 children who had convulsions neonatally One girl was blind due to optic nerve atrophy Three of them had convulsions after the neonatal period and two of these had proven hypoglycaemia post neonatally (7) One developed infantile spasms at 9 months he was severely mentally retarded with cerebral atrophy and spastic diplegia The other was a girl with epilepsy

Pathological EEGs were found in 9 children In group A 2 patients demonstrated generalized dysrhythmia One of them had minimal brain dysfunction (MBD) the oth had a normal status except for the EEG fin ings Five patients from group B showe abnormal EEGs with spikes interpreted a epileptogenic activity Three of these ha epilepsy Patients O E and R D also ha cerebral palsy and their data are presented i Table 2 Patient I L was born at term Th delivery was uncomplicated birth weigh 3 870 g Her condition at birth was good At days of age she became hypotonic with cyano tic attacks and convulsions Blood glucos level was 6 mg/100 ml Her mental develop ment has been normal but she has later experi enced hypoglycaemic episodes and has gran mal epilepsy Another patient in this grou had had febrile convulsions In group C, patients demonstrated theta activity an spikes but none of them had been convulsiv after the neonatal period

Ophthalmological examination revealed op tic nerve atrophy in the blind patien mentioned above A squint was observed in cases In no instance could zonular cataract be detected

The *audiograms* did not disclose hearing de fects in any of the children

Clinical data from 5 patients with *cerebra palsy* are listed in Table 2 Four of them had a birth weight below 2 500 g One group B pa tient had brief hypoglycaemia while pro longed hypoglycaemia and/or convulsions were evident in the others One patient in group C had severe asphyxia at birth following

be associated with hypoglycaemia with possible additional factors dependent on prematurity while in secondary hypoglycaemia such sequelae could presumably be caused by a primary neonatal disorder other than *hypoglycaemia or both*

The neonatal course in these patients has been described in a previous paper (6) Symptomatic transient hypoglycaemia was characterized by diagnosis at a later age hypoglycaemia of long duration and a significantly lower pretreatment blood glucose level than *in patients with asymptomatic* hypoglycaemia These factors were most pronounced among those group B patients who had *neonatal convulsions which could be* taken as a sign of severe and manifest affection *of the CNS* At follow up the most severe sequelae were detected among cases of the latter category Several of these patients were admitted from other hospitals and nurseries often with convulsions Delay in diagnosis probably has worsened the ultimate outcome in these patients

Asymptomatic hypoglycaemia seemed to carry a good prognosis which is in accordance with the findings of several other authors (2 8 9 10 12 13) Patients of this category had short term mild hypoglycaemia during the *first hours after birth The only patient in this* group who had abnormal findings at follow up had initially a very low blood glucose level and continued to show a tendency to hypoglycaemia during the first 5 days of life

The gestational age was lower in group A than in the group B infants seen at follow up (mean 34 5 and 38 3 weeks respectively) Among the group A infants only one out of 7 had a gestation age above 37 weeks while in group B the corresponding figures were 6 out of 9 It thus seems reasonable to assume that CNS damage caused by *prematurity has been* of no significance in this material

Most patients with secondary hypoglycaemia had short term hypoglycaemia without any relation to the occurrence of neonatal convulsions The *findings at follow* up indicate that hypoglycaemia in these patients was of minor importance both with regard to presenting symptoms neonatally and the long term *outcome The CNS damage* documented in these patients tended to be less serious than that caused by the symptomatic transient type of neonatal hypoglycaemia

The frequency of pathological EEG findings (27%) was lower than that recently reported by Pildes and co authors (13) However these *authors performed EEGs yearly in their cases* whereas this was not routinely done in the present series which may account for the discrepancy There were no specific EEG abnormalities associated with hypoglycaemia which was also the case in the patients studied by Pildes et al (13)

In the present series there were a greater number of individuals whose height and weight were below the 25th percentile compared with those exceeding the 75th percentile There were no cases with head circumference below the 2 5 percentile These data are however difficult to interpret as *patient series have not been compared with* a matched control group Pildes and co workers (13) could not demonstrate significant differences in height or weight between patients and controls after the first 2 years of follow up *whereas the head* circumferences of hypoglycaemic groups continued to be significantly smaller *than those of controls at* 2 4 and 6 years of age Koivisto et al (12) found their patients to be subnormal in both height and weight at follow up and there was an increased number of individuals with head *circumference below the 2 5* percentile compared to controls

In conclusion the findings in this *material* are in accordance with those reported by Koivisto et al (12) and by Raivio (15) and indi*cate that duration* of hypoglycaemia and probably also the severity of blood glucose depression are significant *factors in determin*ing the prognosis Awareness of the condition and early treatment of neonatal hypoglycaemia is therefore mandatory

Symptoms	Findings at follow up
Convulsions hypotonia	Mild ataxia squint epilepsy
Convulsions cyanosis	Spastic diplegia infantile spasms epilepsy mental retardation
Hypotonia cyanosis apnoea	Spastic diplegia squint
Cyanosis apnoeic spells	Spastic hemiplegia pathol EEG febrile convulsions
Hypotonia convul sions pH 6 87 (cap)	Mild ataxia

DISCUSSION

Neurological sequelae found at follow up of children with neonatal hypoglycaemia are multiple and range from severe psycho motor retardation to discrete neurological abnormalities such as a pathological electro encephalogram or a squint in otherwise normal children Several authors (2 3 4 10 11, 13) have pointed out the difficulties in in terpreting these findings as neonatal disorders such as prematurity *per se* brain injury or asphyxia occurring concomitantly with hypoglycaemia will of course contribute to cerebral damage in such patients Creery (4) assumed that the prognosis of an infant with idiopathic, symptomatic neonatal hypo glycaemia is better than when the hypo glycaemia results from cerebral birth injury or other severe disturbances Knobloch and co workers (11) found that low birth weight *per se* seemed to play a significant role In their small series Cox & Dunn (3) found no apparent correlation between duration of hypoglycaemia and eventual neurological out come Griffith & Bryant (9) could not demon strate any significant differences at follow up between children who had neonatal hypo glycaemia and matched controls

In most series, reported cases of true or idiopathic, symptomatic neonatal hy[illegible] glycaemia have been presented toget[illegible] with cases where other interfering factors p[illegible] sumably could have contributed to the o[illegible] come This may explain the discrepancy [illegible] findings documented at follow up Koivisto [illegible] al (12) excluded all infants with neonatal d[illegible] orders other than hypoglycaemia that cou[illegible] cause brain injury, and they were then able [illegible] demonstrate a relation between duration [illegible] hypoglycaemia and occurrence of neonat[illegible] convulsions Furthermore children of the la[illegible] ter category more often showed signs of pe[illegible] manent CNS damage than did non convulsi[illegible] patients or those with asymptomatic hyp[illegible] glycaemia Similar findings were reporte[illegible] by Raivio (15) who stated that the duration [illegible] hypoglycaemia was the most important singl[illegible] factor

In the present material the infants were in[illegible] tially classified into three different groups o[illegible] clinical assessment with the intention of ex[illegible] cluding interfering factors as far as possible Thus in infants with asymptomatic and symp tomatic transient hypoglycaemia, CNS dam age documented at follow up could probably

Table 3 *Findings on developmental screening in 37 patients at follow up in neonatal hypoglycaemia*

Category of neonatal hypoglycaemia	No of cases	Results
Asymptomatic	7	6 normal 1 minimal brain dysfunc tion
Symptomatic transient	9	4 normal 2 doubtful 2 minimal brain dysfunct tion 1 severe mental retarda tion
Secondary	21	16 normal 1 doubtful 1 minimal brain dysfunc tion 1 psychomotor retardation 2 delayed speech develop- ment one of whom mentally retarded

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NUTRITION OVERNUTRITION AND OBESITY IN THE FIRST YEAR OF LIFE IN MALMO SWEDEN

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ABSTRACT Sveger T Lindberg T Weibull B and Olsson U L. (Department of Paediatrics Malmo General Hospital Malmo Sweden) Nutrition overnutrition and obesity in the first year of life in Malmo Sweden Acta Paediatr Scand 64 635 1975.—The feeding pattern calorie intake weight and height were studied at various ages during the first 12 months in 243 infants born in Sweden The feeding pattern and calorie intake was close to that recommended 0–6% in each age group were found to be obese (20–40% above the standard weight) and 15–23% overweight (10–20% above the standard weight) The mean calorie intake during the months before and when obesity and overweight were diagnosed exceeded the normal by 10% or less. When re-examined at age 2±½ years 50% of those children obese up to 1 year remained so and only 2 earlier overweight had become obese 25% of the obese children had one obese parent, compared with 10% of the normal children and overweight ones The low incidence of overnutrition and the low freqency of obese and overweight infants in this study compared with previous studies support the idea that high calorie intake is of importance in the development of obesity during infancy Accordingly overnutrition seems to be one factor in the multifactorial aetiology of obesity and reduction of overnutrition can reduce but not abolish infantile obesity Whether the reduction of this will subsequently prevent adult obesity remains to be proved

KEY WORDS Infants obesity overnutrition nutrition

Major changes have occurred in the feeding pattern of Swedish infants Breast feeding has decreased in the western world and milk formulas prefabricated juices and solid foods are used extensively There have been few investigations of the nutrition and calorie intake of Swedish infants (4 7) though recently the nutrition of 4- 8 and 13 year-old children was carefully studied (11) Shukla et al (12) presented an alarmingly high frequency of overnutrition in the first year of life in a field study from England

Obesity has become one of the most prevalent and serious nutritive problems in the western world Since the treatment of obese adolescents and adults has produced remarkably poor results attention has been increasingly directed towards prevention Approximately 80% of juvenile obese children remain obese as adults (6) and they also tend to be the most severely obese and most resistant to treatment (5)

Adipose tissue is suggested to have critical periods of development Brook (2) thinks that a sensitive period occurs from a gestation of about 30 weeks when the adipose tissue begins to grow considerably to the age of one year During these months the basic number of adipose tissue cells is determined subsequently the growth in number of adipose cells becomes resistant to external influences (5) Mossberg (8) suggested two peaks for the onset of juvenile obesity one between birth and 4 years of age another between 7 and 11 According to Eid (3) an excessive gain in weight during the first years of life is of great

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REFERENCES

1 Brown R J & Wallis P C Hypoglycaemia in the newborn *Lancet I* 1 278 1963
2 Cornblath M & Schwartz R Transient symptomatic hypoglycaemia in the neonate *In* M Cornblath & R Schwartz (eds) *Disorders of carbohydrate metabolism in infancy* Saunders Philadelphia and London 1967 p 82
3 Cox M & Dunn H G Idiopathic hypoglycaemia and children of low birth weight *Develop Med Child Neurol 9* 430 1967
4 Creery R D G Hypoglycaemia of the newborn Diagnosis treatment and prognosis *Develop Med Child Neurol 8* 746 1966
5 Eeg-Olofsson O Gentz J Jodal V Nilsson L R & Zetterstrom R Neonatal symptomatic hypoglycaemia Some results from a follow up study *Acta Paediatr Scand* Suppl *177* 85 1967
6 Fluge G Clinical aspects of neonatal hypoglycaemia *Acta Paediatr Scand 63* 876 1974
7 Fluge G Stoa K F & Aarskog D Endocrinological aspects at follow up studies in neonatal hypoglycaemia *Acta Paediatr Scand 64* 280 1975
8 Gentz J Persson B & Zetterstrom R On the diagnosis of symptomatic neonatal hypoglycaemia *Acta Paediatr Scand 58* 449 1969
9 Griffith A D & Bryant G M Assessment of effects of neonatal hypoglycaemia A study of 41 cases with matched controls *Arch Dis Childh 46* 819 1971
10 Haworth J C & McRae K N Neonatal hypoglycaemia A six year experience *Journal Lancet 87* 41 1967
11 Knobloch H Sotos J F Sherard E S Jr Hodson W A & Wehe R A Prognostic and etiologic factors in hypoglycaemia *J Pediatr 70* 876 1967
12 Koivisto M Blanco-Sequeiros M & Karuse U Neonatal symptomatic and asymptomatic hypoglycaemia A follow up study of 151 children *Develop Med Child Neurol 14* 603 1972
13 Pildes R S Cornblath M Warren I Page El E diMenza S Merritt D M & Peeva A A prospective controlled study of neonatal hypoglycaemia *Pediatrics 54* 5 1974
14 Raivio K O Idiopathic hypoglycaemia of childhood manifesting in the neonate *Annales Paediat Fenn 14* 110 1968
15 Raivio K O Factors affecting the development of symptoms in neonatal hypoglycaemia *Annales Paediat Fenn 14* 105 1969

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Table 3 *Mean and standard deviation (S D) of total calorie intake recommended allowances and calorie intake from milk juice and solids in the different age groups*
N=Number of observations MF=Milk formula M=Boys F=Girls

Age (months)	N	Cal/day ±1 S D sample	Cal/kg body weight ±1 S D sample	Mean both sexes	Cal/kg body weight recommended	Cal/day ±1 S D breast milk MF milk sample	Cal/day ±1 S D juice and solids sample	
1	M 67	432± 90	109±20	110	1.0	432± 90	–	
	F 39	441± 69	112±18			441± 69	–	
2	M 86	575± 76	111±18	110	110	571± 76	(38)	13± 7
	F 62	551± 79	109±15			545± 80	(22)	15± 7
3	M 63	652± 84	105±14	106	110	610± 75	(53)	49± 43
	F 58	638± 79	108±16			598± 82	(52)	46± 28
4	M 46	683±145	98±19	98	110	581±114	(43)	109± 98
	F 46	671±123	98±21			575±113	(45)	98± 60
5	M 32	6 0±160	91±17	91	110	454±125		221±140
	F 74	679±125	91±16			432±115		245±121
6	M 34	716± 95	88±12	91	100	395±137		321±109
	F 44	715±125	93±19			364±11?		345±145
7	M 31	763±147	90±17	89	100	348± 97		4?6±135
	F 37	736±1?5	89±14			333±130		403±114
9	M 55	845±143	89±16	88	100	351±130		492±129
	F 41	788±150	86±16			353±116		435±159
12	M 60	925±141	88±16	91	100	285±103		638±135
	F 60	918±170	93±19			307±114		6?1±133

Number of infants in parentheses

months and 3% at 4 months Table 2 summarizes the data.

Bottle feeding

About one third were bottle fed at age 1 month at 4 months 88% were entirely bottle fed (Table 2)

Fruit juice

Fruit juice was introduced at about age 2 months to one third of the infants At age 3 months 94% were given fruit juice Table 3 shows the calorie consumption when the child is given juice and solids

Solid food

The children were not fed solid food before age 2 months It was given in tiny portions to 1/4 of the children at age 3 months and to more than 9/10 at age 4 months

The solids given to the 4 month-old children consisted of factory prepared fruit and vegetable products Factory prepared instant dinners were added at age 4–5 months Few mothers used home-cooked food during the child s first year

Calorie intake

The intake of calories/kg body weight shown in Table 3 was close to the recommended level throughout all the age groups Between age 4 and 6 months the mean calorie con

Table 4 *The percentage of children over weight or obese 0–12 months of age*

Age in months	Total	% Over weight	% Obese
0	235	14	3
1	110	15	4
2	123	18	4
3	119	23	2
4	89	0	6
6	83	?0	0
9	100	15	3
12	1?2	11	6

Table 1 *Composition of milk formulas (MF) Protein fat carbohydrates g/l and calorie value*

Recommended for infants aged (in months)	MF 1 0–5	MF 2 1/2–6	MF 3 More than 4
Calories/l	675	640	650
Protein g/l	15	23	32
Fat g/l	35	25	17
Lactose g/l	70	54	39
Dextrin Maltose glucose g/l	–	23	17
Starch	–	–	32

importance in the prediction of obesity developing in later childhood and is a better guide than the weight of the parents

The present study was made to determine the feeding pattern and the calorie intake the incidence of overnutrition and the relation between the calorie intake and obesity during the first year of life in infants born in Sweden

MATERIAL

During 1971–72 243 babies aged 1–12 months were examined on 885 occasions All were born normally after 38–42 weeks of gestation and included 12 with foreign parents mostly Yugoslavs The infants were examined in two local welfare clinics Each clinic serves the nearby urban population Malmo an industrial town in southern Sweden has about 250 000 inhabitants The distribution of the children by social class was not studied but the clinics probably serve an area with an average social class distribution

The 243 babies represent about 10% of the infants born in Malmo in 1971 They were studied at various ages and on average 4 times at age 1–12 months The variation of the actual age in each age group was ±1 week 213 of the initial 243 were re examined at age 1½–2½ years

METHODS

The examination included a routine clinical check up measurement of length and weight and an interview for the recording of details of the child s diet Body weight was recorded to the nearest 0 010 kg and supine length measured with a measuring table with an accuracy of 0 5 cm Information about height and weight of the parents was obtained from the mother or father at the interview

Nutritive data were collected by the 24 hour recall method (10) Two of us (T S and T L) conducted the interviews The mother or in a few cases the one taking care of the child described the amount of milk formula breast milk fruit juice and solid food given to the child in household measures The breast fed infants were weighed before and after breast feeding to determine the intake The dietary account was recorded on a specially designed questionnaire

Information on calorie intake and height was not obtained in all children (cf Tables 3 and 4)

Calculations of the calorie content of the food were based on manufacturers composition tables Table 1 lists the composition of the different milk formulas The calorie content of breast milk was estimated at 700 cal/l The mothers did not add sugar to the milk and denied using heaped or packed scoops when preparing the formula from milk powder

Obesity was defined by comparing actual weight with that expected for age sex and height (9 12 15) Swedish standard curves were used for comparison (4) The method of calculation was

$$\frac{\text{Actual wt (kg) of infant at present age}}{\text{Actual length (mm) of infant at present age}}=A$$

$$\frac{\text{50th } P \text{ expected wt (kg) for corresp age}}{\text{50th } P \text{ expected length (mm) for corresp age}}=B$$

$A/B\times100$=index for surveyed infant B=standard index taken as equal to 100

The infants were divided into three groups on the basis of the index

1 *Normal* 20% below to 10% above the standard weight index range >80 to ≤110

2 *Overweight* >10–≤20% above the standard weight index range >110 to ≤120

3 *Obese* >20–≤40% above the standard weight index range >120 to ≤140

RESULTS

Breast feeding

The incidence of breast feeding was low At age 1 month 50% were totally breast fed at 2 months 26% at 4 months 9% Mixed feeding was given to 18% at 1 month 16% at 2

Table 2 *Percentage of infants 1–7 months of age having breast milk mixed feeding and the different milk formulas*

Age (months)	Breast milk	Mixed feeding	MF 1	MF 2	MF 3
1	50	18	32	–	–
2	26	16	40	18	–
3	15	11	36	34	4
4	9	3	11	14	63
5	9	2	2	2	85
6	4	5	–	–	91
7	1	2	–	–	97

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	F 41	788±150	86±16			353±116		435±159
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Age in months	Total	% Over weight	% Obese
0	?35	14	3
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2	123	18	4
3	119	23	2
4	89	?0	6
6	83	20	0
9	100	15	3
12	1 2	11	6

Table 5 *Mean calorie consumption of obese and overweight infants during the months before and when the excess weight was diagnosed compared with mean of all the surveyed babies*

N=Number of observations

Group	Age in months 1 (Cal/day)	2 (Cal/day)	3 (Cal/day)	4 (Cal/day)
Overweight and obese	462 (N=38)	610 (N=31)	690 (N=31)	748 (N=22)
Total sample	436	565	645	677

sumption/kg body weight was 12–19 calories/kg below the standard recommended by the Swedish National Institute of Public Health (13) and at age 7–12 months about 10 calories/kg below In these age groups however 18–26% were overweight or obese (Table 4) Calorie intake calculated in relation to the normal weight for the actual length shows it to be approximately that recommended

Breast milk and the milk formulas supplied almost 100% at age 3 months 83% at 4 months 53% at 5 months 43% at 9 months and 32% at 12 months Table 3 gives the total calorie intake calories supplied by milk juice and solid food

At age 9 months 84 of 96 children got about 2/3 of the calories supplied by milk from milk formula 3 (MF3) and 1/3 from standard milk At age 1 year 95 of 120 got 1/2 of the milk calories from MF and 1/2 from standard milk The remaining children had only standard milk

The mean calorie intake of the 23 obese infants, the months before and the month when by definition they were obese was about 10% above the mean of all the surveyed infants The number of observations in each age group to age 6 months was 7–10 This is too small for statistical analyses Table 5 lists the calorie consumption of obese *and* overweight children 1–4 months of age The number of observations in the other age groups was too small for reliable statistical analyses

Infantile obesity

The curve of weight and length of the surveyed boys and girls ran parallel to the standard curve between mean and +1 S D from age 3–12 months

Fig 1 shows the mean weight and length in the age groups for the girls plotted in the Swedish standard curves Fig 2 *a* and *b* gives the mean weight of the overweight and obese children Their mean length did not show any difference from the normal surveyed infants

The incidence of obesity in the various age groups was 0–6% a further 15–23% were overweight as shown in Table 4 Sex did not seem to influence the frequency of obesity before age one year At age 12 months however 16% boys and 5% girls were overweight and 8% boys and 4% girls were obese

Three who were by definition obese only

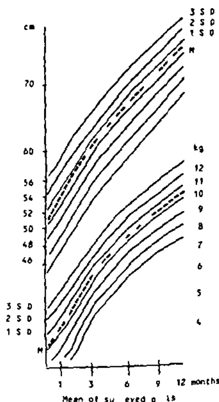


Fig 1 Mean weight and length for the surveyed girls (– – –) compared with Swedish standard curves (Mean (M) ± 3 S D)

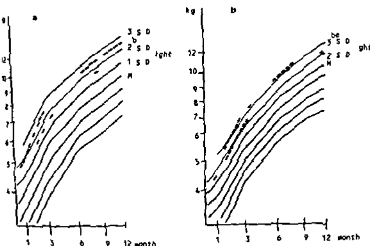


Fig 2 Mean weight (---) of the overweight and obese (*a*) boys and (*b*) girls compared with Swedish standard curves (Mean (M) ±3 SD)

at birth are included in the obese group The mean birth weight of the 12 obese boys was 4 786 g and of the 11 obese girls 4 031 g The birth weight of the obese is significantly ($p<0.01$) above that of the total sample 25% of the obese children had one obese parent none had both

At age 1½–2½ years 213 of 243 were re examined The frequency of obesity in this age group was 5% Two who were overweight be fore age one year had become obese No obese child came from the normal weight group 18 of 23 obese infants have so far been studied at age 2 ±½ years 9 of these were still obese The other 9 had become normal or overweight at about age 2 years and these were all obese only during their first 6 months of life

DISCUSSION

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The calorie intake is higher in the already referred to English study (12) compared with ours (see Fig 3) It could be because solid food is introduced later in Sweden This prob ably contributes slightly to the total calorie intake in Swedish children being smaller than that of the English

During the first year of life 0–6% were obese and another 15–23% overweight in the different age groups This percentage should be compared with 16 7% and 27 7% respec tively observed by Shukla et al (12) A simi lar high frequency was reported by Eid (3) The total proportion of overweight and obese children in our material 17–26% was also considerably lower than the 44% in the Eng

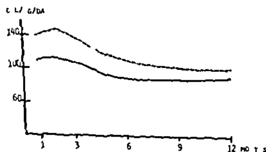


Fig 3 The mean calorie intake per kg body weight and day of the surveyed children (—) compared with the ap proximate mean in the study by Shukla et al (12) ( )

Table 5 *Mean calorie consumption of obese and overweight infants during the months before and when the excess weight was diagnosed compared with mean of all the surveyed babies*

N=Number of observations

	Age in months			
Group	1 (Cal/ day)	2 (Cal/ day)	3 (Cal/ day)	4 (Cal/ day)
Overweight and obese	462 (*N*=38)	610 (*N*=31)	690 (*N*=31)	748 (*N*=22)
Total sample	436	565	645	677

sumption/kg body weight was 12–19 calories/kg below the standard recommended by the Swedish National Institute of Public Health (13) and at age 7–12 months about 10 calories/kg below In these age groups however 18–26% were overweight or obese (Table 4) Calorie intake calculated in relation to the normal weight for the actual length shows it to be approximately that recommended

Breast milk and the milk formulas supplied almost 100% at age 3 months 83% at 4 months 53% at 5 months 43% at 9 months and 32% at 12 months Table 3 gives the total calorie intake calories supplied by milk juice and solid food

At age 9 months 84 of 96 children got about 2/3 of the calories supplied by milk from milk formula 3 (MF3) and 1/3 from standard milk At age 1 year 95 of 120 got 1/2 of the milk calories from MF and 1/2 from standard milk The remaining children had only standard milk

The mean calorie intake of the 23 obese infants, the months before and the month when by definition they were obese was about 10% above the mean of all the surveyed infants The number of observations in each age group to age 6 months was 7–10 This is too small for statistical analyses Table 5 lists the calorie consumption of obese *and* overweight children 1–4 months of age The number of observations in the other age groups was too small for reliable statistical analyses

Infantile obesity

The curve of weight and length of the surveyed boys and girls ran parallel to the standard curve between mean and +1 S D from age 3–12 months

Fig 1 shows the mean weight and length in the age groups for the girls plotted in the Swedish standard curves Fig 2 *a* and *b* gives the mean weight of the overweight and obese children Their mean length did not show any difference from the normal surveyed infants

The incidence of obesity in the various age groups was 0–6% a further 15–23% were overweight as shown in Table 4 Sex did not seem to influence the frequency of obesity before age one year At age 12 months however 16% boys and 5% girls were overweight and 8% boys and 4% girls were obese

Three who were, by definition obese only

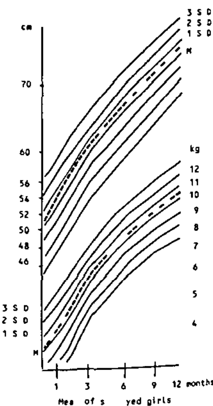


Fig 1 Mean weight and length for the surveyed girls (– – –) compared with Swedish standard curves (Mean (*M*) ± 3 S D)

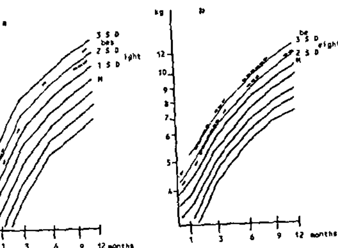


Fig 2 Mean weight (- - -) of the overweight and obese (*a*) boys and (*b*) girls compared with Swedish standard curves (Mean (M) ±3 S D)

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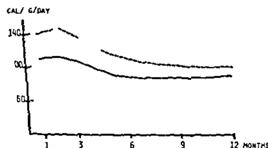


Fig 3 The mean calorie intake per kg body weight and day of the surveyed children (—) compared with the approximate mean in the study by Shukla et al (12) ( )

lish studies The Swedish and English standard curves for weight and length are comparable (4 14)

The English study with a high calorie intake and high frequency of obesity, when compared with the Swedish with a normal calorie intake and a low frequency of obesity shows a correlation between high calorie intake and obesity On the other hand the obese and overweight children s mean calorie consumption in our survey was only slightly increased compared with the mean intake of the sample

The obese infants had a considerably higher birth weight This was also reported by Shukla et al (12) but was not noted by Wolff (15) Our results indicate a constitutional tendency towards obesity 25% of the obese children, age 0–2½ years had one obese parent none had two Earlier studies have shown that 40–50% of the children with one obese parent will develop obesity (5) The environmental factors are difficult to separate from the genetic The prevalence of obesity among parents of normal and overweight babies was 5%

What could be done by nutritive means to further reduce the frequency of obesity during the first year of life? The idea that these infants are obese because they eat too much is to over simplify the problem The energy consumption varies from child to child and a low energy consumption can also contribute to the development of obesity Moreover a child is probably born with a certain range of adipose tissue cellularity which could be influenced by a variety of environmental factors The final size of the adipose tissue will depend upon the interaction between a genetic template and environmental and hormonal factors that influence the number and size of the adipose cells as suggested by Knittle (5) The calorie intake should be carefully adjusted to an amount normal in relation to the infant s calculated normal weight The substantial normal variation in the intake of calories must be avoided Even then it seems inevitable that there will be a number of obese infants

REFERENCES

1 Beal V A The nutritional history in longitudinal research *J Am Diet Assoc 51* 426 1967

2 Brook C G D Evidences for a sensitive period in adipose cell replication in man *Lancet II* 624 1972

3 Eid E E Follow up study of physical growth of children having excessive weight gain in the first 6 months of life *Br Med J II* 74 1970

4 Karlberg P Engstrom I Lichtenstein H & Svennberg I The development of children in a Swedish urban community *Acta Paediatr Scand 57* Suppl 87 1968

5 Knittle J L Obesity in childhood A problem in adipose tissue cellular development *J Pediatr 81* 1048 1972

6 Lloyd J K Wolff O H & Whelen W S Childhood obesity long term study of height and weight *Br Med J II* 45 1961

7 Mellander A Vahlquist B & Mellbin T Breast feeding and artificial feeding *Acta Paediatr Scand 48* Suppl *116* 1959 p 116

8 Mossberg H O Obesity in children a clinical prognostical investigation *Acta Paediatr Scand 35* Suppl 2 1948

9 Newens E M & Goldstein H Height weight and the assessment of obesity in children *Br J Prev Soc Med 26* 33 1972

10 Pekkarinen M Rivioja S & Jortikka L A comparison of the food intake of rural families estimated by one-day recall and precise weighing methods *Voeding 28* 470 1967

11 Samuelson G An epidemiological study of child health and nutrition in a northern Swedish county *Acta Paediatr Scand 60* Suppl *214* 1971

12 Shukla A Forsyth H A Anderson Ch M & Marrian S M Infant overnutrition in the first year of life A field study in Dudley Worcestershire *Br Med J IV* 507 1972

13 Swedish National Institute of Public Health *Var Foda 21* 167 1969

14 Tanner J M Whitehouse R H & Takaishi M Standards from birth to maturity for height weight height velocity and weight velocity British Children 1965 *Arch Dis Child 41* 613 1966

15 Wolff O H Obesity in childhood *Q J Med 24* 109 1955

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CHEMICAL PATHOLOGY OF KRABBE S DISEASE

III *Ceramide hexosides and Gangliosides of Brain*

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ABSTRACT Vanier M Th and Svennerholm L (Department of Neurochemistry Psychiatric Research Centre University of Goteborg Goteborg Sweden) Chemical pathology of Krabbe s disease III Ceramide-hexosides and gangliosides of brain Acta Paediatr Scand 64 641 1975.—Neutral ceramide-hexosides and gangliosides in cerebral cortex and white matter of children who had died in Krabbe s disease were quantitatively isolated and characterized The concentrations of galactosylceramides lactosylceramides and glucosylceramides were normal or slightly increased in cerebral cortex but all the three glycolipids were diminished in white matter particularly the galactosylceramides More complex ceramide hexosides globotriose globotetraose and blood-group substance H present in trace amounts in normal brain were much more abundant in cerebral cortex and especially in white matter of brains affected by Krabbe s disease The composition of the ceramide portion suggested that these glycolipids as well as a portion of the lactosylceramides and glucosylceramides were structural components of the globoid cells. The ganglioside distribution was severely altered G_D and G_M were severely reduced in cerebral cortex and white matter while G_{D1b} and G_T were slightly decreased in cerebral cortex but increased in white matter Normally minor brain gangliosides metabolically related to $G_{D\,b}$ and G_T i e G_{D3} G_{D2} and G_{M3} were strongly increased in cerebral cortex and in white matter

For the first time galactosylsphingosine (psychosine) was demonstrated in normal infant brain. In cerebral tissue affected by Krabbe s disease the concentration of psychosine was at least 10 times higher The large increase in this cytotoxic substance might be the primary lesion in Krabbe s disease

KEY WORDS Psychosine fatty acids sphingosine

The concept of Krabbe s disease as an inborn error of sphingolipid metabolism is now well established and a diminished activity of galactosylceramide β galactosidase is assumed to be the primary enzymatic defect (19) Krabbe s disease however differs from other sphingolipidoses despite a metabolic block in the degradative pathway of galactosylceramide there is no apparent accumulation of this lipid in brain or other organs because the storage is restricted to the nervous tissue and to a particular cell type—the globoid cells One of the salient features is instead a severe decrease of galactosylceramide in white matter On the other hand several neutral glycolipids unrelated to galactosylceramide traces of which occur in normal brain have been described as large fractions (3 14) Severe changes in the ganglioside patterns have also been found (3 23) The disturbance of the sphingolipid metabolism in Krabbe s disease has therefore many facets and a systematic investigation of the glycolipid fractions should provide useful information about the pathogenesis of the condition

Table 1 *Concentration of neutral glycosylceramides and gangliosides of cerebrum in Krabbe's disease*

Values are expressed in nanomoles/g fresh tissue weight

	Cerebral cortex				White matter			
	Normal 2–27 months	Krabbe's disease 7–32 months I	II	III	Normal 2–27 months	Krabbe's disease 7–32 months I	II	III
Neutral glycolipids								
Galactosylceramide	975	650		780	21 400	3 700		3 600
Glucosylceramide	5	16		17	110	32		91
Lactosylceramide	30	36		52	273	140		150
Globotriose	<1	8		26	<1	40		6[illegible]
Globotetraose	1	13		14	1	64		100
Blood group subst H	<1			8	<1	15		19
Gangliosides	1 328	1 101	1 060		735	707	805	
G_{M3}	30	59	84		22	79	168	
G_{D3}	41	114	50		20	100	149	
G_{M2}	84	106	110		60	66	67	
G_{D2}	22	65	84		19	83	62	
G_{M1}	438	279	258		373	109	138	
G_{D1a}	502	354	292		172	182	123	
G_{D1b}	106	84	85		35	52	64	
G_{T1}	103	40	97		32	36	54	

We have quantitatively isolated all glycolipids and gangliosides of cerebral tissue in a large series of Krabbe's disease. The quantitative isolation procedure permitted accurate determination of the ceramide portion of these glycolipids. A preliminary report has been published earlier (27).

MATERIAL AND METHODS

Chemicals

The same chemicals were used as for the isolation of the same lipids in normal infant brain (31).

Brain material

Specimens of brain material were obtained at autopsy of 16 patients who had died of Krabbe's disease between the ages of 7 and 32 months. The brain lipid composition in cerebral cortex and white matter was characteristic of the disease (33, 34). Cerebral cortex and white matter were carefully dissected. Four different preparations were made with specimens from 6–10 different brains in each batch. The size of the samples varied between 15 and 350 g cerebral cortex and 25 and 140 g white matter.

Methods

The general scheme elaborated from the isolation of sphingolipids of normal infant brain was followed (13, 22, 24, 25, 31, 32). A detailed description of the separation procedure and the chemical characterization of the isolated glycolipids and gangliosides will be given elsewhere.[1]

The concentrations of neutral glycolipids and gangliosides were determined by analysis on the isolated fraction, that of sialic acid by the resorcinol procedure (21, 22), that of lipid hexose with the orcinol method (20) and that of fatty acids by GLC with the fatty acid 21 0 as internal standard.

For analysis of the ceramide portion, samples containing 100–300 nmoles of lipid were hydrolysed in methanol–water–conc HCl 10 1 1 (by vol) for 16 h at 73 (5). The methyl esters of the fatty acids were analysed by GLC on DEGS columns operated at 190 (28, 31). The sphingosines were oxidized to their corresponding aldehydes (29) and analysed by GLC with the same procedure as that used for normal infant brain (31).

RESULTS

Glycolipid concentration

Neutral glycolipids (Table 1). Galactosylceramide (galactocerebroside) constituted approximately 700 nmol/g in cerebral cortex, a value close to that found in the control brain. In the white matter its concentration was 3 600 nmol/g, i.e. only 17% of that in agematched controls. The concentration of

[1] Vanier M T, Månsson J E & Svennerholm L, in preparation.

Table 2 *Patterns of cerebral gangliosides in Krabbe s disease*

Values are expressed in molar percentage

	Cerebral cortex				White matter			
	Controls V-11 4-?7 months		Krabbe s disease N= 1 7-32 months		Controls N=11 4-27 months		Krabbe s disease N=19 7-32 months	
Ganglioside	Mean	S D	Mean	S D	Mean	S D	Mean	S D
G_{M3}	3 8	1 8	7 7	3 1	4 3	1 8	13 3	4 0
G_{D3}	3 -	1 1	4 8	0 7	3 7	2 3	11 9	3 0
G_{M2}	6 6	2 6	12 0	4 7	7 2	9	9 2	3 4
G_{D2}	3 1	1 1	7 6	2 1	? 3	1 1	6 9	1 9
G_{M1}	29 5	4 2	27 5	5 0	45 8	5 8	25 4	5 1
G_{D1a}	37 7	3 3	4 1	4 6	?6 5	5 4	16 0	4 8
G_{D1b}	7 7	1 7	7 9	1 6	5 3	1 9	9 0	2 6
G_{T1}	7 7	1 2	7 0	1 3	4 4	1 2	6 5	2 1
G_Q	0 8	0 2	1 ?	0 4	0 6	0 2	1 6	0 7

glucosylceramide in cerebral cortex was higher than that in the control brains but showed a reduction in the white matter

As in normal brain lactosylceramide was the second largest glycolipid both in cerebral cortex and in white matter In cerebral cortex its concentrations was slightly larger than in normal infant brain but in white matter it was only about 50% of that in the controls A glycolipid migrating on TLC distinctly slower than lactosylceramide of normal brain was isolated from white matter Its concentration was 13 nmol/g Analysis of the alditol acetates and sequential hydrolysis by specific hydrolases revealed the uncommon structure Galβ →Gal→Cer This fraction contained exclusively hydroxy fatty acids

A small amount of galactosylsphingosine (psychosine) was isolated both from cerebral cortex and white matter in the latter it constituted 10 *nmol/g fresh weight In a normal* 4 month old infant cerebral white matter its concentration was less than 1 *nmol/g*

Higher ceramide hexosides were markedly increased Globotriose Galα(1→4)Galβ(1→4) Glc–Cer and globotetraose GalNAcβ(1→3) Galα(1→4)Galβ(1→4)Glc–Cer which constituted at most 2 nmol/g in the normal infant brain were large fractions their concentrations ... were constantly higher in white matter than in cerebral cortex Another fraction migrating on TLC as gangliotetraose G_{A1} was a mixture of several ceramidehexosides in which blood group substance H predominated

Gangliosides (Tables 1 and 2) Compared with what was found in the age matched controls *the ganglioside concentration was decreased* in cerebral cortex and slightly increased *in white matter of brains from patients* with Krabbe s disease

In cerebral cortex *the major gangliosides* were decreased the concentrations of G_{M1} and G_{D1} were moderately *reduced while those of* G_{D1b} and G_{T1} were only slightly reduced Statistical treatment *of the relative percentages* of gangliosides found in individual analytical samples showed *only G_{D1a} to be* significantly diminished Another feature was a prominent increase in »minor» gangliosides a 2 to 3 fold increase of G_{M3} and G_{D3} a 3 to 4 fold increase of G_{D2} and a slight increase of G_{M2} Though their determination is less accurate in small samples significant changes in these gangliosides were found also on analysis of ganglioside patterns in the individual samples (Table 2)

Still larger changes were observed in the *white matter* The most striking findings were a severe reduction of G_{M1} to about 1/3 of nor

Table 3 *Fatty acid composition of neutral glycosylceramides of cerebrum in Krabbe s disease*
Mean values of results obtained from determinations of preparations I and III expressed in molar percentage
C C = cerebral cortex W M = white matter

Compound		Fatty acid[a] 16 0	18 0	20 0	22 0	23 0	24 0	24 1	>24C
Glycosylceramide	C C	7	40	4	8	10	17	11	3
	W M	6	24	3	10	13	23	17	3
Lactosylceramide	C C	10	52	3	5	4	9	11	5
	W M	8	27	3	7	8	18	22	5
Triglycosylceramide	C C	7	51	3	8	4	8	17	1
	W M	6	55	3	6	4	6	19	1
Globotetraose	C C	6	40	3	9	4	10	24	1
	W M	4	50	3	6	4	6	25	1
Unknown	W M	3	51	3	6	4	6	26	1

[a] Fatty acids 16 1 18 1 22 1 23 1 were also detected but constituted less than 1%

mal and a strong increase of G_{M3} and G_{D3}, which gangliosides constituted larger proportions than G_{M1} and G_{D1a} and thus became the dominating fractions G_{D2} was increased to a level close to that in cerebral cortex while G_{M2} had quite a normal concentration An unexpected finding was an increase in G_{D1b} and G_{T1} Compared with normal age matched controls the relative distribution of all gangliosides except that of G_{M2} was significantly increased or diminished

Fatty acid composition

Neutral glycosylceramides (Table 3) In all the neutral glycolipids two types of fatty acids predominated stearic acid and C$_{4}$ acids Stearic acid constituted between 40 and 55% of all fractions except of glucosylceramide and

Table 4 *Fatty acid composition of gangliosides of cerebrum in Krabbe s disease*
Mean values of results obtained from determinations of preparations I and III expressed in molar percentage
C C = cerebral cortex W M = white matter

Compound		Fatty acid 16 0	18 0	20 0	22 0	23 0	24 0	24 1
G_{M3}	C C	6	70	4	5	3	4	8
	W M	16	46	4	6	6	8	14
G_{D3}	C C	3	73	5	4	2	4	9
	W M	3	47	3	9	5	9	26
G_{M2}	C C	3	88	6	1	1	1	1
	W M	3	88	5	1	1	1	1
G_{D2}	C C	1	89	6	1	1	2	<1
	W M	1	76	5	3	3	3	9
G_{M1}	C C	2	89	6	1	1	1	<1
	W M	2	86	4	2	1	2	4
G_{D1}	C C	1	91	5	<1	<1	1	1
	W M	1	92	6	<1	<1	<1	<1
G_{D1b}	C C	2	87	6	1	1	1	2
	W M	2	62	5	4	3	5	19
G_{T1}	C C	1	88	6	1	1	1	2
	W M	2	69	5	3	3	5	13

[a] Fatty acids 16 1 18 1 22 1 23 1 and >24C were also detected but constituted less than 1%

le 5 Sphingosine composition of neutral osylceramide and gangliosides of cere n in Krabbe s disease

es are expressed in molar percentage
=cerebral cortex W M =white matter

npound		Sphingosine		
		d18 0	d18 1	d 0 1
tral glycolipids				
cosylceramide	C C	6	94	-
	W M	4	93	3
ctosylceramide	C C	4	88	8
	W M	4	93	3
glycosylceramide	C C	13	87	-
	W M	5	94	-
obotetr ose	W M	5	91	2
nknown	W M	4	95	-
anglios des				
M3	C C	5	86	9
	W M	4	90	6
D3	C C	5	80	15
	W M	5	87	7
M2	C C	4	79	17
	W M	6	76	18
G D2	C C	6	63	31
	W M	4	72	[illegible]6
G M	C C	4	76	[illegible]0
	W M	4	77	19
G D1	C C	3	76	[illegible]1
	W M	3	73	[illegible]4
G b	C C	3	73	[illegible]4
	W M	4	82	14
G_T	C C	3	71	[illegible]6
	W M	3	78	18

lactosylceramide of white matter These two glycolipids had fatty acid compositions which differed in several other respects Both in cerebral cortex and white matter 3–5% of the acids had a chain length longer than 24 carbon atoms The ratio 24 0/24 1 and the propor tion of 23 0 were also higher in these glycolipids than in the others particularly in glucosylceramide

Globotriose and globotetraose had closely related patterns but the proportion of stearic acid tended to be somewhat larger in white matter The fatty acid pattern of blood group substance H was identical with that of globotetraose Hydroxy fatty acids were found in galactosylceramides and sulfatides but not in any of the other glycosylceramides except in digalactosylceramide The propor tions of the major hydroxy acids in the latter were 22h 0 10% 23h 0 12% 24 0 59% and 24h 1 8%

Gangliosides (Table 4) Stearic acid was the dominating fatty acid of all the gangliosides Fatty acids with a chain length above C_{24} never constituted more than 1% In cerebral cortex all the gangliosides except G_{M3} and G_{D3} had very similar patterns with almost exclu sively 18 0 and 20 0 fatty acids G_{M3} and G_{D3} contained larger amounts of 22 0 23 0 24 0 and particularly 24 1 In the white matter only G_{M2} G_{M1} and G_{D1a} had a fatty acid composition similar to that found in cortex G_{M3} G_{D3} G_{D1b} and G_{T1} had much larger proportions of 22 0 23 0 and 24 0 and above all 24 1 A large content of 16 0 was invariably found in G_{M3} from different preparations

Sphingosine composition (Table 5)

Sphingosines were analysed to determine the ratio between sphingosines with 18 and 20 carbon atoms and minor amounts of other sphingosines if any were not recorded A feature common to all neutral glycolipids and psychosine was an extreme predominance of sphingosine with 18 carbon atoms *d* 20 1 sphingosine constituted at most 3% except in lactosylceramide of cerebral cortex in which it made up 8%

In cerebral cortex of patients with Krabbe s disease sphingosine *d* 20 1 was 20 to 26% of total in the normally major brain gangliosides G_{M1} G_{D1a} G_{D1b} and G_{T1} In G_{M2} and G_{D3} it constituted only 15–17% while in G_{M3} it con stituted less than 10% The highest content of sphingosine *d* 20 1 was found in G_{D2}

In the white matter the sphingosine pat terns of G_{M2} G_{M1} and G_{D1a} were fairly similar to those of cerebral cortex Gangliosides G_{M3} G_{D3} G_{D1b} and G_{T1} had a significantly lower content of *d* 20 1 sphingosine in white matter than in grey matter

DISCUSSION

Several previous studies on Krabbe s disease have been concerned with the neutral

glycolipids and gangliosides of brain (1 3 4 14, 16 23) The results are difficult to interpret and their biological significance is limited for several reasons

(i) there has been no appropriate parallel study of a relevant control brain material

(ii) although glycolipids and gangliosides are classified from their carbohydrate structure they have often been characterized only by chromatography of the intact lipids and the sequential arrangement of the sugars has been determined in only relatively few substances

(iii) the amounts of brain material from individual cases have been so small as to incur the risk that physiologically important substances occurring in very low concentrations e g psychosine might have escaped detection

(iv) the ceramide portion has been analysed in only few glycolipids

Glucosyl and lactosylceramides were approximately increased two fold in the cerebral cortex but reduced to about 50% in the white matter The latter finding is incompatible with earlier observations (3 4 14 16 23) but in the previous studies the normal levels in white matter were considered to be very low The concentrations of glucosyl and lactosylceramides of normal human infant brain are however much higher in cerebral white matter than in cortex (31) their fatty acid patterns resemble that of galactosylceramide (26 31) which suggests that they are formed in the same metabolic compartment and are thus related to the myelination process In Krabbe s disease, the fatty acid patterns of glucosyl and lactosylceramide have also similarities with those of globotriose and globotetraose which support the idea that portions of glucosyl and lactosylceramides might be structural components of the globoid cells It was Eto & Suzuki (3) who first suggested that globotriose and globotetraose were components mainly of the globoid cells rather than of the neural tissue The fatty acid compositions of these glycolipids in the present study corroborate this assumption Our detection of blood group substance H in brains of patients with Krabbe s disease is also in line with this view On TLC this ceramide hexoside migrates like gangliotetraose G_{A1} and it seems that it has previously been erroneously identified as G_{A1} by Eto & Suzuki (3) and by us (27) Quantitative determination of the hexoses in the ceramide dihexoside fraction always showed a galactose glucose ratio slightly higher than 1 0 All attempts to separate digalactosylceramide, if any from lactosylceramide failed The isolation of a digalactosylceramide with hydroxy fatty acids alone suggests, however that the lactosylceramide fraction also contained a small portion of digalactosyl ceramide with normal fatty acids

Changes in the gangliosides, particularly of those in the cerebral white matter, have been reported previously (1 3, 16 23 27) In the present study the ganglioside patterns showed large inter individual variations particularly in the concentrations of G_{M3} and G_{D3} which often became the two major gangliosides in the white matter Although a very large number of brains from patients with Krabbe's disease have been studied we have been unable to find any correlation between the increase of these gangliosides and the severity of the other biochemical changes or the duration of the disease A large increase in G_{M3} G_{D3} and G_{D2} has been described in several other demyelinating diseases such as subacute sclerosing panencephalitis (10 17) metachromatic leukodystrophy (17 23) and recently in plaques of multiple sclerosis patients (35) The study of the ceramide portion of G_{M3} and G_{D3} in normal brains has suggested that they were synthesized in another metabolic compartment (31) A feature common to all these diseases and globoid cell leukodystrophy is a severe gliosis G_{M3} and G_{D3} are assumedly located in proliferating mesenchymal cells On the other hand a constant finding in the white matter was the decrease of G_{M1} This finding was consequent with the knowledge that G_{M1} is a major component of human myelin gangliosides (7 11) and that

there is an almost complete lack of myelin in brains of patients with Krabbe s disease Its concentration was however still lower than expected from myelin content alone (35)

An important finding was a 10-fold increase in the amount of galactosyl sphingosine (psychosine) The primary genetic lesion in Krabbe s disease is assumed to be a defect of cerebroside β galactosidase (19) One of the unanswered questions however is why galactocerebroside does not accumulate either in brain if one excludes the small amounts stored in the globoid cells or in extra neural organs (16 18) [2] In brain the striking feature is a very lipid poor white matter with signs of delayed maturation (33 34) Miyatake & Suzuki (15) have suggested that galactosylceramide might be converted to galactosylsphingosine which could be very cytotoxic (15 30) In a recent report Lin & Radin (12) were however unable to demonstrate any degradation of galactosylceramide to psychosine Psychosine can also be synthesized from sphingosine and UDP galactose (2 6 8) Our present finding of the normal occurrence of this lipid in small amounts and its increase in Krabbe s disease again raises the question of the physiological significance of this reaction A possible mechanism of the pathogenesis of Krabbe s disease which would also explain its exclusive localization to the nervous system could be as follows the residual activity of cerebroside β galactosidase is sufficient to effect the degradation of the small amounts of galactosylceramide present in extra neural organs In brain the oligodendroglial cell synthesize not only galactosylceramide but also galactosylsphingosine Galactosylceramide is built into the plasma membrane of the cell while psychosine is not and is normally rapidly degraded In Krabbe s disease galactosylsphingosine is degraded at a much lower rate than normal (15) its concentration increases and soon reaches a toxic level with the result that the oligodendroglial cells die and myelination stops

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[2] Svennerholm L & Vanier M T unpublished results

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REFERENCES

1 Berra B & Zambotti V Patterns of brain ganglioside fatty acids in sphingolipidoses *Adv Exp Med Biol* 25 311 1972
2 Cleland W W & Kennedy E P The enzymatic synthesis of psychosine *J Biol Chem* 235 45 1960
3 Eto Y & Suzuki K Brain sphingolipids in Krabbe s globoid cell leukodystrophy *J Neurochem* 18 503 1971
4 Evans J E & McCluer R H The structure of brain dihexosylceramide in globoid cell leukodystrophy *J Neurochem* 16 1393 1969
5 Gaver R C & Sweeley C C Methods for methanolysis of sphingolipids and direct determination of long-chain bases by gas chromatography *J Am Oil Chem Soc* 42 294 1965
6 Hildebrand J Stoffyn P & Hauser G Biosynthesis of lactosylceramide by rat brain preparation and comparison with the formation of ganglioside G_{M1} and psychosine during development *J Neurochem* 17 403 1970
7 Kamoshita S Aron A M Suzuki K & Suzuki M Infantile Niemann Pick disease A chemical study with isolation and characterization of membranous cytoplasmic bodies and myelin *Am J Dis Child* 117 379 1969
8 Kanfer J N Incorporation of C UDP glucose and ¹⁴C UDP galactose into carbohydrate-containing sphingolipids by a rat brain particulate fraction *Lipids* 4 163 1969
9 Karlsson K A Sphingolipid long chain bases *Lipids* 5 878 1970
10 Ledeen R W Salsman K & Cabrera, M Gangliosides in subacute sclerosing panencephalitis isolation and fatty acid composition of nine fractions *J Lipid Res* 9 129 1968
11 Ledeen R W Yu R K & Eng L F Gangliosides of human myelin sialosylgalactosylceramide (G_7) as a major component *J Neurochem* 21 829 1973
12 Lin Y N & Radin N S Alternate pathways of cerebroside catabolism *Lipids* 8 732 1973
13 Mårtensson E Öhman R Graves M & Svennerholm L Galactosyltransferases catalysing the formation of the galactosyl–galactosyl linkage in glycosphingolipids *J Biol Chem* 249 4132 1974
14 Menkes J H Duncan C & Moossy J Molecular composition of the major glycolipids in globoid cell leukodystrophy *Neurology* 16 581 1966

Miyatake T & Suzuki K Globoid cell leukodystrophy additional deficiency of psychosine galactosidase *Biochem Biophys Res Commun 48* 538 1972

Philippart M Glycolipid mucopolysaccharide and carbohydrate distribution in tissues plasma and urine from glycolipidoses and other disorders Complex nature of the accumulated substances *Adv Exp Med Biol 25* 231 1972

Suzuki K Ganglioside patterns of normal and pathological brain *In* S M Aronson & B W Volk (eds) *Inborn disorders of sphingolipid metabolism* Pergamon Press Oxford 1967 pp 169–186

— Renal cerebroside in globoid cell leukodystrophy (Krabbe s disease) *Lipids 6* 433 1971

Suzuki K & Suzuki Y Globoid cell leucodystrophy (Krabbe s disease) deficiency of galactocerebroside β galactosidase *Proc Natl Acad Sci USA 66* 302 1970

Svennerholm L The quantitative estimation of cerebrosides in nervous tissue *J Neurochem 1* 42 1956

— Quantitative estimation of sialic acids II A colorimetric resorcinol-hydrochloric acid method *Biochim Biophys Acta 24* 604 1957

— The distribution of lipids in the human nervous system I Analytical procedure Lipids of foetal and newborn brain *J Neurochem 11* 839 1964

— The metabolism of gangliosides in cerebral lipodoses *In* S M Aronson & B W Volk (eds) *Inborn disorders of sphingolipid metabolism* Pergamon Press Oxford 1967 pp 169–186

— Isolation of gangliosides *In* R L Whistler & M L Wolfrom (eds) *Methods in carbohydrate chemistry* vol 6 Academic Press New York 1972 pp 464–474

Svennerholm L Månsson J E & Li Y T Isolation and structural determination of a novel ganglioside a disialosylpentahexosylceramide from human brain *J Biol Chem 248* 740 1973

Svennerholm L & Stallberg Stenhagen S Changes in the fatty acid composition of cerebrosides and sulfatides of human nervous tissue with age *J Lipid Res 9* 215 1968

27 Svennerholm L & Vanier M Th Brain gangliosides in Krabbe s disease *Adv Exp Med Biol 19* 499 1972

28 — The distribution of lipids in the human nervous system III Fatty acid composition of glycerophospholipids of human foetal and infant brain *Brain Res 50* 341 1973

29 Sweeley C C & Moscatelli E A Quantitative microanalysis and estimation of sphingolipid bases *J Lipid Res 1* 40 1959

30 Taketomi T & Nishimura K Physiological activity of psychosine *Jap J Exp Med 34* 255 1964

31 Vanier M Th Holm M Månsson J E & Svennerholm L The distribution of lipids in the human nervous system V Gangliosides and allied neutral glycolipids of infant brain *J Neurochem 21* 1375 1973

32 Vanier M Th Holm M Öhman R & Svennerholm L Developmental profiles of gangliosides in human and rat brain *J Neurochem 18* 581 1971

33 Vanier M Th & Svennerholm L Chemical pathology of Krabbe s disease I Lipid composition and fatty acid patterns of phosphoglycerides in brain *Acta Paediatr Scand 63* 494 1974

34 — Chemical pathology of Krabbe s disease II Fatty acid composition of cerebrosides sulfatides and sphingomyelins in brain *Acta Paediatr Scand 63* 501 1974

35 Yu R K Ledeen R W & Eng L F Ganglioside abnormalities in multiple sclerosis *J Neurochem 23* 169 1974

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CHEMICAL PATHOLOGY OF KRABBE S DISEASE

IV Studies of Galactosylceramide and Lactosylceramide β-Galactosidases in Brain White Blood Cells and Amniotic Fluid Cells

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ABSTRACT Svennerholm L Håkansson G and Vanier M Th (Department of Neurochemistry Psychiatric Research Centre University of Goteborg Goteborg Sweden) Chemical pathology of Krabbe s Disease IV Studies of galactosylceramide and lactosylceramide β-galactosidases in brain white blood cells and amniotic fluid cells. Acta Paediatr Scand 64 xxx 1975.—Galactosylceramide β-galactosidase and lactosylceramide β-galactosidase activities were investigated in normal human brain leukocytes and amniotic fluid cells. The enzymatic assays were performed on brains from 11 patients with Krabbe s disease on leukocytes from 16 patients and 18 obligate heterozygotes and on amniotic fluid cells from 9 foetuses at risk The brain enzyme was solubilized from a 900 g–100 000 g pellet. With this enzyme preparation a profound deficiency of galactosylceramide β-galactosidase activity in brain approximately 1% of that in age-matched controls was shown The lactosylceramide β-galactosidase activity of brain was also strongly reduced but not to the same extent as the other β-galactosidase Galactosylceramide β-galactosidase activity in leukocytes from patients with Krabbe s disease was generally less than 5% of that in age-matched controls, and there was no overlap between the patients and the obligate heterozygotes Carrier detection by the leukocyte enzyme was however not possible because of considerable overlap between heterozygotes and normal controls The lactosylceramide β-galactosidase activity was only moderately reduced in leukocytes but strongly reduced in cerebral tissue from patients with Krabbe s disease The changes in the glycolipid pattern of cerebral tissue recently described by us in patients with Krabbe s disease offers an explanation to the serious glycolipid β-galactosidase deficiency in CNS

KEY WORDS Krabbe s disease particulate enzyme carrier detection

A profound deficiency of galactosylceramide β galactosidase activity was demonstrated by Suzuki and co-workers (14) in brain liver and spleen of three patients with Krabbe s disease In a later study (9) they showed that also galactosylsphingosine β galactosidase was deficient in the disease and Wenger and co workers (26) reported a marked deficiency of monogalactosyldiglyceride β galactosidase There is evidence that the same enzyme may act on all the three galactolipids (10 26) Wenger et al (27) recently also reported a profound deficiency of lactosylceramide β galactosidase of brain liver and fibroblasts in two patients with Krabbe s disease and considered the possibility of a storage of lactosylceramide in brain which could lead to an early delay of myelination a characteristic feature of Krabbe s disease Since we had autopsy material from carefully clinically examined patients with Krabbe s disease in whom we had made extensive determinations of the brain lipid composition we decided to try to elaborate simple and reliable enzymic

methods for the diagnosis of Krabbe s disease We also planned to study the glycolipid storage in brain and the activity of glycolipid hydrolases for any correlation in an attempt to elucidate the physiopathological mechanisms Finally we wished to use the same enzymic methods for the early diagnosis of Krabbe s disease, and if possible also for the detection of carriers of the disease

MATERIAL AND METHODS

Enzymes

Brain tissue to be used as a control material was obtained at autopsy from children who had died in accidents or in a few cases of diseases with no history of metabolic disorder Autopsy specimens were also obtained from patients who had died of Krabbe s disease The diagnosis was verified by pathological anatomical examination and by biochemical determinations of lipids (24) These tissues were stored frozen for less than 3 years at −20 and in general less than 1 year

The enzyme was prepared in the following way 0 5 g of tissue was homogenized in 5 ml of 0 32 M sucrose in 0 01 M Tris buffer pH 7 4 in a small glass homogenizer with rotating knives (MSL) at maximum speed for 1 min The homogenate was further homogenized in an all glass Potter Elvehjem apparatus with 6 strokes at 1200 rpm The homogenate was centrifuged as before The resulting pellet was discarded and the combined supernatants were centrifuged at 100 000 g for 60 min The supernatant was discarded and the pellet was re suspended in 1 ml of glass distilled water The homogenate was frozen thawed and sonicated for 1 min in a water bath type ultrasonicator (Cleanet 50 Compact 75W Gneshaberg AG Zurich) The treatment was repeated 3 times after which the tube was centrifuged at 40 000 g for 60 min The supernatant was used as the enzyme The yield of enzyme protein was 2–5% of total protein of the brain specimen All operations were performed at +4

Leukocytes were obtained from controls (healthy adult males and females and children with various neurological diseases except Krabbe s disease) patients with Krabbe s disease and obligate heterozygotes of Krabbe s disease 10 ml of blood was drawn in a tube with 0 5 ml of 0 1 M EDTA and 2 5 ml of 6% dextran (Macrodex Pharmacia) Leukocytes were isolated after differential sedimentation for 90 min Contaminating red cells were lysed with ammonium chloride The white cells were re suspended in 1 ml of 0 9% NaCl and a portion was taken for counting The white cell sample was frozen thawed and ultrasonicated for 1 min

Amniotic fluid cells The amniotic fluid cell cultures were terminated for assay after 4 weeks They were entirely or predominantly fibroblastic in morphology at the time of harvest The cells were collected after 3–5 min exposure to pronase (15 PUK/ml physiological saline) washed twice with 0 9% NaCl suspended in a suitable volume of 0 9% NaCl frozen thawed and ultrasonicated prior to assay

Substrates

Galactosylceramide was prepared from human cerebral white matter The substance gave two bands at thin layer chromatography (TLC) but did not contain any glucose Lactosylceramide was isolated from human spleen by TLC (19) or prepared from human brain gangliosides by mild acid hydrolysis (0 1 M HCl at 100° for 1 h) Ganglioside G_{M1} was isolated by column chromatography of crude brain gangliosides (20) treated by sialidase at pH 7 2 and saponified The purified G_{M1} was then isolated by renewed chromatography on silicic acid The two glycolipids were made radioactive with tritium in the terminal galactosyl residue by the method of Radin et al (12) The radioactive glycolipids were purified by column chromatography on Sephadex G 25 and silicic acid and finally by TLC on silica gel G developed by chloroform–methanol–water 65 25 4 or 60 32 7 (by vol) Enzymatic hydrolysis with β galactosidase (6) revealed that 99% of the radioactivity was present in the terminal galactosyl residue

Chemicals

The following commercial chemicals were used for this study 4 methyl-umbelliferyl β D galactoside 4 methyl umbelliferyl-N acetyl β D glucosaminide and sodium taurocholate pure from Koch Light Laboratories Colnbrook Bucks England silica gel H and G for column and thin layer chromatography of natural substrates Fluka AG Buchs Switzerland Sephadex G 25 superfine and Macrodex Pharmacia Uppsala Sweden galactose oxidase Kabi AB Stockholm Sweden sodium[^{3}H]-borohydride Radiochemical Centre Amersham England Instagel Packard Instrument Co Downers Grove Ill neuraminidase from Vibrio cholerae Behringwerke Marburg Lahn BRD pronase E 70 000 PUK/g Merck AG Darmstadt BRD Tris base (Trizma) Sigma Chemical Co St Louis Mo bovine serum albumin (Fraction V) BDH Chemicals Ltd Poole Dorset England

Assay methods

N acetyl β glucosaminidase and β galactosidase activities were assayed with 4 methylumbelliferyl β-glycosides β galactosidase according to van Hoof & Hers (22) and glucosaminidase according to Hultberg & Öckerman (4) Galactosylceramide and lactosylceramide β galactosidase activities were assayed with methods based on the previous experience in the laboratories of Drs Brady (5) Radin (2) and Suzuki (14 17)

Galactosylceramide β galactosidase was assayed in an incubation mixture which consisted of 0 1 ml substrate in 0 2 M sodium acetate buffer (pH 4 2) and enzyme diluted to 0 3 ml with glass-distilled water The substrate consisted of 50 nmol labelled galactosylceramide (10 000 dpm/nmol) and 0 9 mg sodium taurocholate uniformly suspended by ultrasonication The enzyme consisted of 25–50 μg protein of white matter from controls and 40–120 μg protein of cerebral cortex and white matter from cases of Krabbe s disease Of white blood cells and amniotic cells 20–100 μg enzyme protein was used The blank tubes contained 0 3 ml of water instead of enzyme After incubation for 2 hrs at 37 the reaction was stopped

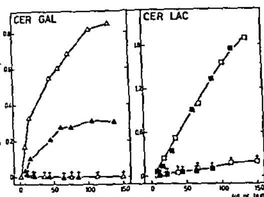


Fig 1 Effect of protein concentration on β-galactosidase activities Galactosylceramide β-galactosidase in cerebral cortex ▲ normal ▲ Krabbe s disease in white matter △ normal △ Krabbe s disease lactosylceramide β-galactosidase in cerebral cortex ■ normal ■ Krabbe s disease in white matter □ normal □ Krabbe s disease The activities of the enzymes were assayed according to the standard procedures described in the text except that the protein concentration varied

by placing the tube in an ice bath and adding 0 2 ml bovine serum albumin and 1 5 ml ice cold 10% trichloroacetic acid The tubes were left in an ice bath for 5 min and centrifuged for 10 min at 1500 g in a refrigerated centrifuge A 1 0 ml aliquot of the supernatant was added to 10 ml Instagel and the radioactivity was determined by liquid scintillation spectrometry Corrections for quenching by the trichloroacetic acid were made with the automatic external standard technique

The assay method for lactosylceramide β galactosidase was elaborated in the same way 50 nmol labelled lactosylceramide (10000 dpm/nmol) and 2 0 mg sodium taurocholate were uniformly suspended by ultrasonication in 0 1 ml 0 2 M sodium acetate buffer pH 4 2 and 0 3 ml of enzyme diluted with glass-distilled water The following protein concentrations were used cerebral cortex and white matter of controls and cases of Krabbe s disease 10–100 µg protein white cells and amniotic cells 20–100 µg The tubes with the reaction mixture were incubated for 30 min at 37 after which the reaction was stopped by placing the tube in ice-cold water and adding 0 2 ml 10% serum albumin and 1 5 ml ice-cold 10% TCA The released galactose was determined as in the method for galactosylceramide β-galactosidase

Protein was assayed by the method of Lowry et al (7) with crystallized and lyophilized fatty acid free bovine serum albumin as standard

RESULTS

The optimal conditions for the assay of cerebroside β galactosidase and lactosylceramide β galactosidase were determined with enzyme preparations of normal human brain and white blood cells

Enzyme concentration

With the method adopted for the isolation of the enzyme galactosylceramide β galactosidase activity was generally linear up to 20 µg protein in white matter and 50 µg protein of cerebral cortex in 0 4 ml (Fig 1) The lactosylceramide β galactosidase activity was linear up to 200 µg protein/0 4 ml in both cerebral cortex and white matter

In the brain specimens from cases of Krabbe s disease galactosylceramide β galactosidase activity was almost invariably highest at the lowest protein concentrations used and then dropped to zero at 100 µg protein/0 4 ml (Fig 1) The activity was thus approximately 0 1 nmol/2 h when 5–10 µg protein was tested The lactosylceramide β galactosidase activity was also significantly diminished in brains of cases of Krabbe s disease but showed a linear response up to 200 µg protein/0 4 ml (Fig 1)

Increasing concentrations of enzyme protein of brains affected with Krabbe s disease were added to 25 µg enzyme protein of white matter and 50 µg protein of cerebral cortex of control brains to a volume of 0 4 ml A slight and linear decrease in galactosylceramide activity occurred with increasing amounts of enzyme protein from the brains with Krabbe s disease and the activity was reduced to

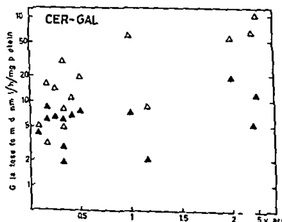


Fig 2 Specific activity of galactosylceramide β-galactosidase in normal human brain at different ages Symbols are as in Fig 1

methods for the diagnosis of Krabbe s disease We also planned to study the glycolipid storage in brain and the activity of glycolipid hydrolases for any correlation in an attempt to elucidate the physiopathological mechanisms Finally we wished to use the same enzymic methods for the early diagnosis of Krabbe s disease and if possible also for the detection of carriers of the disease

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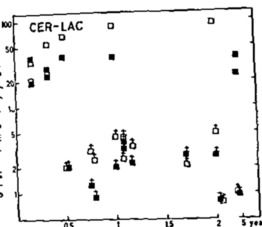


Fig 4 Specific activity of lactosylceramide β-galactosidase in normal human brains and brains from patients with Krabbe s disease at different ages Symbols are as in Fig 1

value only slightly lower than that of an obligate carrier

Amniotic fluid cells The normal controls showed only a narrow range of variation between 4–5 nmoles of galactose was formed per hour per mg protein In the 9 samples of cells from 9 families in which a child with Krabbe s disease had been born the variation was wide and it was not possible to distinguish between carriers and healthy homozygotes just as it was impossible to distinguish between carriers and homozygotes in leukocytes The lowest value found in this study was 1 6 nmoles/h/mg protein which is more than 30% of the mean value found for the controls

Lactosylceramide β-galactosidase

Brain The lactosylceramide activity of the control brains increased with age and was 2–3 times as high in white matter as in cerebral cortex (Fig 4) In brains from patients with Krabbe s disease the activity was equal in cerebral cortex and white matter and did not vary with age The activity was 5–10% of that in the control brains

Leukocytes The normal activity of lactosylceramide β galactosidase showed the same variation as cerebroside β galactosidase (Table 1) In patients with Krabbe s disease the activity was approximately 40% of that found in the normal controls

DISCUSSION

Miyatake & Suzuki (11) recently argued that the use of purified enzyme preparations carries *its own risk in the study of inherited* metabolic disorders The purification procedure might lead to elimination of fractions which contain the crucial abnormality We were guided by similar considerations in the present study and for more than two years we have tried to handle crude preparations of human brains for the assay of different glycolipid β galactosidases in controls and patients with *Krabbe s disease With a crude homogenate or* a low speed supernatant of human cerebrum a reproducible specific activity of galactosylceramide β galactosidase was obtained when the amount of enzyme protein used was kept constant When we used the same amount of protein as Suzuki & Suzuki (14) similar results were obtained for the cerebroside β galactosidase activity of normal brain The activity varied strongly also with the concentration of the endogenous substrate

Our method for the isolation of a purified enzyme was based on the procedure used by Miyatake & Suzuki (8) for the isolation of galactosylsphingosine galactosylhydrolase When Wenger and co workers (26) used the same procedure for the assay of galactosylceramide β galactosidase they observed that the 15 000 *g* supernatant fraction discarded by Miyatake & Suzuki (8) contained high enzyme activity We therefore made our enzyme from the 900 *g* to 100 000 *g* pellet This enzyme preparation was found to be an excellent source for the assay of galactosylceramide and lactosylceramide β galactosidases of normal brain and brains from patients with Krabbe s disease The partially purified enzymes showed the same behaviour as those of a crude preparation they had the same optimal pH

Table 1 *Activities of galactosylceramide and lactosylceramide β-galactosidases in leukocytes of controls and in patients and obligate heterozygotes for Krabbe s disease (measured in nmoles galactose formed per hour per 10⁶ cells)*

Enzyme activity	Controls Adults N	Mean	S D	Krabbe s disease Patients N	Mean	S D	Parents N	Mean	S D
N acetyl β glucosaminidase	(61)	276	71	(16)	257	97	(18)	309	116
Galactosylceramide β galactosidase	(55)	0 25	0 11	(16)	0 01	0 01	(18)	0 15	0 13
Range		(0 11–0 50)			(0 00–0 03)			(0 04–0 33)	
Lactosylceramide β galactosidase	(17)	0 81	0 35	(4)	0 30	–	(4)	0 50	–
Range		(0 41–1 46)			(0 20–0 48)			(0 4?–0 64)	

approximately 75% after the addition of 100 μg enzyme protein from brains affected with Krabbe s disease No such diminution of activity was found when leukocyte enzyme of patients with Krabbe s disease was added to control leukocytes The lactosylceramide activity of control brains was not affected by the addition of enzyme protein of brains affected with Krabbe s disease either

Galactosylceramide β-galactosidase

Brain The enzyme was essentially free from endogenous substrate and gave a high value for galactosylceramide β galactosidase activity of white matter Fig 2 gives the galactosylceramide β galactosidase activities expressed as nmoles galactose formed per hour per mg protein of normal cerebral cortex and white matter from term to 5 years of age From two months of age the activity was approximately twice as high in white matter as in cortex, this difference increased and became 10 times as large in white matter as in cortex from 2 years of age In the cerebral cortex and white matter of 10 cases of Krabbe s disease, less than 0 05 nmoles of galactose was formed from cerebroside per hour per mg protein when 100 μg enzyme protein was used in the test This means that the galactosylceramide β galactosidase activity was less than 1% of that in cerebral cortex as well as in white matter of the age matched controls

Leukocytes The galactosylceramide β galactosidase activity of white blood cells was equal in children and adults—0 25 nmoles of galactose was formed per hour per million cells which corresponds to 1 36 nmol/h/mg protein (Table 1 Fig 3) It is evident from Fig 3 that the normal variation is considerable and one could postulate from the values in the controls that diagnosis of carriers is impossible Fig 3 also shows that the activity values of controls and carriers overlap to a large extent The mean values for the patients 0 015 nmol/h/million cells was 6% of the mean values for the controls and only one patient had a

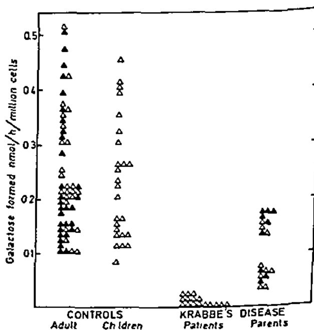


Fig 3 Galactosylceramide β galactosidase activities in leukocytes △ adult females and children ▲ adult males

confirm Suzuki s previous results that all patients with Krabbe s disease had a significant reduction of galactosylceramide β galactosidase in leukocytes (15 16) But leukocytes cannot be used for detecting carriers Several obligate heterozygotes had normal values Suzuki et al (15 16) have proposed that blood serum should be used for the detection of carriers Because the activity of galactosylceramide β galactosidase in serum is very low and the assay has to be performed on fresh serum we disagree with Suzuki and cannot recommend serum for this purpose in Scandinavia

Suzuki et al (13) reported less than 5% of the normal galactosylceramide β galactosidase activity in amniotic fluid cells in a pregnancy in which there was a risk for Krabbe s disease and in which the diagnosis was confirmed by electron microscopical and biochemical analysis of the aborted foetus (3) We have assumed that fetuses with Krabbe s disease should have less than 20% of the normal galactosylceramide β galactosidase activity in their amniotic fluid cells Of our 9 samples of amniotic fluid cells from women who had already given birth to a child with Krabbe s disease the activity of this galactosidase never was lower than 30% of the mean control value We regarded all of them as healthy and the pregnancies were not interrupted It is however a serious source of uncertainty that so far only one case of Krabbe s disease has been diagnosed in amniotic fluid cells and that the fibroblasts of patients with Krabbe s disease have a higher residual activity of galactosylceramide β galactosidase (15) than all other tissue materials studied This means that the lower limit of the range for foetal carriers of Krabbe s disease can only be arbitrary

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REFERENCES

1 Austin J Suzuki K Armstrong D Brady R Bachhawat B K Schlenker J & Stumpf D Studies in globoid (Krabbe) leukodystrophy (GLD) V Controlled enzymic studies in ten human cases *Arch Neurol 23* 502 1970

2 Bowen D M & Radin N S Cerebroside galactosidase a method for determination and a comparison with other lysosomal enzymes in developing rat brain *J Neurochem 16* 501 1969

3 Ellis W G Schneider E L McCulloch J R Suzuki K & Epstein C J Fetal globoid cell leukodystrophy (Krabbe disease) *Arch Neurol 29* 253 1973

4 Hultberg B & Öckerman P A Artificial substrates in the assay of acid glycosidases *Clin Chim Acta 39* 49 1972

5 Kanfer J N Young O M Shapiro D & Brady R O The metabolism of sphingomyelin I Purification and properties of a sphingomyelin cleaving enzyme from rat liver tissue *J Biol Chem 241* 1081 1966

6 Li Y T & Li S C Anomeric configuration of galactose residues in ceramide trihexosides *J Biol Chem 246* 3769 1971

7 Lowry O H Rosebrough N J Farr A L & Randall R J Protein measurement with the Folin phenol reagent *J Biol Chem 193* 265 1951

8 Miyatake T & Suzuki K Galactosylsphingosine galactosylhydrolase partial purification and properties of the enzyme in rat brain *J Biol Chem 247* 5398 1972

9 — Globoid cell leukodystrophy additional deficiency of psychosine galactosidase *Biochem Biophys Res Commun 48* 538 1972

10 — Galactosylsphingosine galactosylhydrolase in rat brain probable identity with galactosylceramide galactosyl hydrolase *J Neurochem 22* 231 1974

11 — Glycosphingolipid β galactosidase in rat brain properties and the standard assay procedures of the enzymes in whole homogenate *Biochim Biophys Acta 337* 333 1974

12 Radin N S Hof L Bradley R M & Brady R O Lactosyl ceramide galactosidase comparison with other sphingolipid hydrolases in developing rat brain *Brain Res 14* 497 1969

13 Suzuki K Scheider E L & Epstein C J In utero diagnosis of globoid cell leukodystrophy *Biochem Biophys Res Commun 45* 1363 1971

14 Suzuki K & Suzuki Y Globoid cell leukodystrophy (Krabbe s disease) deficiency of galactocerebroside β galactosidase *Proc Natl Acad Sci USA 66* 302 1970

15 Suzuki K Suzuki Y & Fletcher T F Further studies on galactocerebroside β-galactosidase in globoid cell leukodystrophy *Adv Exp Med Biol 19* 487 1972

16 Suzuki Y & Suzuki K Krabbe s globoid cell leukodystrophy deficiency of galactocerebrosidase in serum leukocytes and fibroblasts *Science 171* 73 1971

and optimal detergent concentrations [1] Lactosylceramide β galactosidase showed a lower pH optimum than that (pH 5 0) previously reported for rat brain (12) but it was the same as that recently found by Miyatake & Suzuki (11) The developmental curve of lactosylceramide β galactosidase activity showed a variation with age the distribution between cerebral cortex and white matter resembled that of galactosylceramide β galactosidase but differed from that of G_{M1}-ganglioside β galactosidase We have previously shown (23) that the concentration of lactosylceramide was approximately 10 times higher in cerebral white matter than in cerebral cortex of a child s brain Lactosylceramide of white matter also had a ceramide composition similar to that of the galactosylceramide portion with normal fatty acids This similarity suggested a common compartment for the biosynthesis of galactosylceramide and lactosylceramide of cerebral white matter (23) The lactosylceramide β galactosidase of white matter can be assumed specifically to hydrolyse this lactosylceramide and not the lactosylceramide which is an intermediate substance in the degradation of gangliosides

Suzuki and co workers (14 15 16) demonstrated in Krabbe s disease a genetically deficiency of the β galactosidase which hydrolyses galactosylceramide In a later study they showed that the degradation of galactosylsphingosine (psychosine) was also insufficient and Wenger and co workers (26) reported a deficient hydrolysis of mono galactosyldiglyceride A single enzyme probably catalyses the catabolism of these three glycolipids (10 26) Austin et al (1) reported that lactosylceramide β galactosidase was slightly increased in Krabbe s disease while Wenger and co workers (27) recently found an extremely low activity of this β galactosidase in liver brain and cultured fibroblasts from 2 patients with Krabbe s disease The present study showed that the lactosylceramide β galactosidase activity was severely diminished in Krabbe s disease but not to the extent reported by Wenger et al (27)

We do not think that there is a generalized primary deficiency of lactosylceramide β galactosidase in Krabbe s disease, but assume that not only cerebroside β galactosidase but also the other glycolipid β galactosidases are affected in Krabbe s disease Experimental support for such an assumption has recently been produced by Suzuki & Suzuki (18) In our study the lactosylceramide β galactosidase activity was in fact only reduced to 40% of the control value in leukocytes The much more pronounced diminution of lactosylceramide β galactosidase in brain than in other tissues is also shared by galactosylceramide β galactosidase which had less than 1% residual activity in brain compared with 6% in leukocytes The very low activity in brain can be explained by our recent finding of a high concentration of galactosylsphingosine (psychosine) in brains from patients who had died from Krabbe s disease (25)

Galactosylceramide and lactosylceramide β galactosidases are biosynthesized in the same cell compartment Because of the deficiency of galactosylceramide β galactosidase the tissue concentration of psychosine will increase A toxic level of this strongly cytoxic agent (9 21) will be reached early and the machinery for the biosynthesis and biodegradation of galactosylceramide and lactosylceramide will be destroyed This could explain two prominent features in Krabbe s disease viz the early delay in myelination and the extensive reduction of galactosyl ceramide and lactosylceramide β galactosidase activities in brain

The other major aim of the study was to find out whether determination of cerebroside β galactosidase of white blood cells or serum was a reliable tool for the enzymatic diagnosis of Krabbe s disease and could also be used for detecting carriers of the disease We could

[1] Svennerholm L Håkansson G and Vanier M Th (in preparation)

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SHORT COMMUNICATION

CHROMOSOME STUDIES IN PREMATURE LOW BIRTH WEIGHT INFANTS

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Since the W H O Expert Group on Prematurity (1) endorsed the international definition and recommended that an immature infant is a liveborn infant with a weight of 2 500 g or less it has been recognized that this category has a dual population i e there are small babies born at term and those born after a short gestation period Among many factors that have been implicated in the etiology of low birth weight in man an association between chromosome anomalies and full term low birth weight has been presented (2 3) The present communication is a sequel to our previous report (3) and deals with chromosome analyses in a group of premature low birth weight neonates (PLBWN)

The PLBWN (newborns who weighed 2 500 g or less with a gestation period of less than 38 weeks) were selected from all newborn babies delivered at the Georgia Baptist Hospital in Atlanta Georgia between September 1 1969 and August 31 1971 From the same population pool a control group of normal birth weight neonates was chosen by matching them with the study group for sex maternal age and race

Among the 8 542 babies (white 4 072 male 3 849 female black 297 male 324 female) delivered during the two year period of this study there were 413 PLBWN No blood was available from 3 PLBWN Therefore chromosome analyses were carried out on the cord blood of 410 PLBWN of whom 360 were white (207 male 153 female) and 50 were black (25 male 25 female) The chromosomes of the same number of controls were examined

With the use of conventional chromosome analysis method (3) we found no chromosome anomalies in the 410 PLBWN On the other hand 2 chromosome disorders (one white 47 XXX and one white 47 XXY) were observed in the controls Statistically the incidence of chromosome aberrations between the study group and controls is not significant ($p>0$ 25) It appears therefore that while chromosome abnormalities can account for a proportion of full term low birth weight infants (2 3) they do not play a role in the etiology of low birth weight babies born prematurely

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REFERENCES

1 World Health Organization Expert group on prematurity (Final report) *Wld Hlth Org Tchn Rep Ser* No 27 1950

17 — Glycosphingolipid β galactosidases I Standard assay procedures and characterization by electrofocusing and gel filtration of the enzymes in normal human liver *J Biol Chem 249* 2098 1974
18 — Glycosphingolipid β galactosidases II Electrofocusing characterization of the enzymes in human globoid cell leukodystrophy (Krabbe s disease) *J Biol Chem 249* 2105 1974
19 Svennerholm E & Svennerholm L The separation of neutral blood serum glycolipids by thin layer chromatography *Biochim Biophys Acta* 432 1963
20 Svennerholm L Isolation of gangliosides *In* R L Whistler and M L Wolfrom (eds) *Methods in carbohydrate chemistry* Academic Press New York 1972 vol 6 pp 464–474
21 Taketomi T & Nishimura K Physiological activity of psychosine *Jap J Exp Med 34* 255 1964
22 Van Hoof F & Hers H G The abnormalities of lysosomal enzymes in mucopolysaccharidoses *Europ J Biochem* 7 34 1968
23 Vanier M T Holm M Månsson J E & Svennerholm L The distribution of lipids in the human nervous system V Gangliosides and allied neutral glycolipids of infant brain *J Neurochem 21* 1375 1973
24 Vanier M Th & Svennerholm L Chemical pathology of Krabbe s disease I Lipid composition and fatty acid patterns of phosphoglycerides in brain *Acta Paediatr Scand 63* 494 1974
25 — Chemical pathology of Krabbe s disease III Ceramide hexosides and gangliosides of brain *Acta Paediatr Scand 64 1974*
26 Wenger D A Sattler M & Markey S P Deficiency of monogalactosyl diglyceride β galactosidase activity in Krabbe s disease *Biochem Biophys Res Commun 53* 680 1973
27 Wenger D A Sattler M & Hiatt W Globoid cell leukodystrophy deficiency of lactosylceramide β galactosidase *Proc Natl Acad Sci USA 71* 854 1974

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CASE REPORT

CARDIORESPIRATORY SYNDROME DUE TO ENLARGED TONSILS AND ADENOIDS

A Case Report with Discussion Regarding Medical Treatment and Pathogenesis

B THANOPOULOS D D IKKOS M MILINGOS and D FOUTAKIS

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ABSTRACT Thanopoulos B Ikkos D D Milingos M and Foutakis, D (Haemodynamic Laboratory and the Paediatric Department Piraeus General Hospital Nikea Piraeus Greece) Cardiorespiratory syndrome due to enlarged tonsils and adenoids. Acta Paediatr Scand 74 659 1975.—A case of cardiorespiratory syndrome secondary to chronic upper airway obstruction from hypertrophied tonsils and adenoids involving a three-year-old boy is presented The haemodynamic changes were verified by heart catheterization The possible role of steroids in the treatment of the syndrome is suggested

KEY WORDS Cardiorespiratory syndrome airway obstruction hypertrophic tonsils and adenoids.

Reversible cor pulmonale secondary to upper airway obstruction from hypertrophied tonsils and adenoids was first described by Noonan in 1965 (24) The syndrome is relatively rare and there have been a total of 50 cases in the literature (1 3 4 7 9 10 17 18 20 24 25 26 27 28) Diagnosis of this syndrome is of paramount importance since its potential lethal sequelae can be prevented by early tonsillectomy and adenoidectomy

We would like to report a case involving a 3 year old boy with congestive heart failure associated with enlarged tonsils and adenoids and discuss the pathogenesis and the value of steroid treatment in the management of this syndrome

CASE REPORT

A 3 year-old boy was transferred to our clinic with the diagnosis of probable nephrotic syndrome Since the age of 8 months he was noted to have progressively increased respiratory difficulty stertorous breathing especially during sleep and frequent upper respiratory infections

Physical examination on admission disclosed the following adenoid facies and noisy breathing The temperature was 37 8°C heart rate 150/min and respiratory rate 60/min Blood pressure was 100/60 mmHg (right arm) The tonsils and adenoids were huge and obstructed the oropharynx almost completely There were suprasternal and intercostal retractions and few scattered rhonchi and rales The second heart sound was split with accentuation of the pulmonic component No murmur was heard There was slight pitting edema of the lower extremities and the liver was felt 5 cm below the right costal margin Neurological examination was normal except for some irritability

Laboratory findings Hb 13 4 g/100 ml hct 39% The electrocardiogram showed sinus tachycardia left axis deviation P pulmonale incomplete right bundle branch block (IRBBB) and right ventricular hypertrophy (RVH) (Fig 1*a*) The chest roentgenogram revealed cardiomegaly and increased hilar shadows (Fig 2*a*)

Blood gases obtained from a venous sample showed a pH of 7 39 P_{O_2} of 42 mmHg a P_{CO_2} of 41 mmHg oxygen saturation of 78% and a serum bicarbonate of 23 mEq/l The remaining laboratory studies were normal The boy

2 Chen A T L Sergovich F R McKim J S Barr M L & Gruber D Chromosome studies in full term low birth weight mentally retarded patients *J Paediatr 76* 393 1970
3 Chen A T L Falek A & Lester W Chromosome aberrations in full term low birth weight neonates *Humangenetik 21* 13 1974

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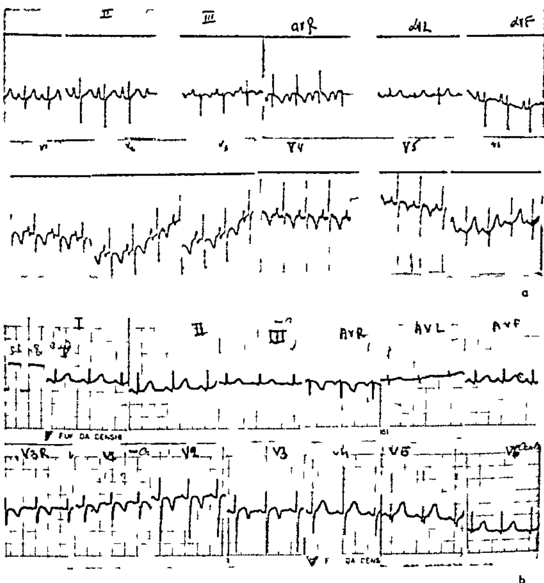


Fig 1 Electrocardiograms of the patient *(a) Before (b)* After operation

was treated with digoxin diuretics and antibiotics Following admission to the hospital he was observed to have marked respiratory difficulty mainly while sleeping when he sometimes awoke struggling for breath Hydrocortisone was added to the treatment i e 100 mg given intravenously every 8 hours for 2 days He responded well to this treatment and after 24 hours was able to sleep more comfortably Five days later the clinical picture had improved considerably The pedal edema had disappeared and the liver was barely palpable

A cardiac catheterization was performed 20 days after admission under general anesthesia using endotracheal intubation It revealed severe pulmonary hypertension associated with high right ventricular systolic and end diastolic pressures and no evidence of intracardiac shunts (Table 1) The angiographic studies ruled out abnormalities of the pulmonary arteries Both ventricles had reduced myocardial contractility One hundred percent oxygen administration for 10 minutes resulted in moderate reduction of the pulmonary arterial and right and left atrial pressures Later while the patient was breathing room air these pressures remained low

Following completion of the procedure and extubation of the patient he immediately developed severe cyanosis and bradycardia associated with complete heart block Arterial blood gases and pH obtained at that time showed pH 7 28 P_{O_2} 45 mmHg P_{CO_2} 57 mmHg oxygen saturation 68 % The child was re intubated given oxygen Isuprel bicarbonate and recovered completely within a few minutes

Three days later the patient underwent tonsillectomy and adenoidectomy The respiratory difficulty disappeared immediately after the operation Four months later the child appeared to be in good health happy and had gained three pounds The electrocardiogram and the chest roentgenogram at this time were normal (Figs 1*b* 2*b*)



Fig 2 Chest roentgenograms of the patient (*a*) Before (*b*) After remo al of tonsils and adenoids

DISCUSSION

In 1965 Noonan (24) and later Menashe et al (21) for the first time described a syndrome of reversible cor pulmonale associated with chronic upper airway obstruction due to hypertrophied tonsils and adenoids Since then at least 50 cases of this syndrome have been reported in the literature Other causes such as laryngotracheomalacia (6) Pierre Robin syndrome (17) laryngeal haemaetoma (20) and macroglossia (25) have been associated with this syndrome and Freeman in 1973 (8) reported its occurrence in cases of congenital heart disease

The clinical picture includes adenoid facies stertorous breathing respiratory difficulty (especially during sleep) somnolence and occasionally mental retardation (3 25) There is also a history of recurrent upper airway infections In more advanced cases congestive heart failure may appear and at times the disease may be lethal (1) The mean age at the onset of symptoms is 2–4 years with a range of 0–9 years

The chest roentgenogram has shown pulmonary edema and cardiomegaly and the electrocardiogram P pulmonale with RVH and IRBBB An unexplained left axis deviation was found in the initial electrocardiogram of our case which returned to normal 4 months following the operation Cardiac catheterization data include pulmonary hypertension elevated right heart chamber pressures with marked respiratory variation and high left atrial and wedge pressures Hypercapnia and acidosis are common findings

The exact pathophysiological mechanism has not been completely clarified It has been shown that obstruction of the upper airway mainly during inspiration may cause alveolar hyperventilation resulting in hypoxia and hypercapnia (4 5 13) This is often exaggerated during sleep because of physiological hypoventilation (23) Chronic hypoxia in susceptible persons (hyper reactors) leads to pulmonary vasoconstriction and results in

Table 1 *Cardiac catheterization data*

Catheter position	O_2 saturation (%)	Pressure in mmHg: O_2 100% administration: Initial	After 10 min	Room air
S V C	75			
R A	78	*a*=22 Mean=17	*a*=13 Mean=11	*a*=13 Mean=9
R V	74	85 / 10– 0 Mean=45	60 / 4–13	65 / 8–13
P A	74	85 / 30	60 / .8	65 / 30
Wedge		Mean=17		
L A		Mean=18	Mean=12	Mean=14
Aorta	95			95 / 55 Mean=70

pulmonary hypertension (11, 16), which may be further aggravated by hypercapnia and acidosis (2) The normal P_{CO_2} values in the venous blood of our patient suggest absence of hypercapnia while awake However, cases without or minimal hypercapnia have been reported (3 4)

With regard to the etiology of pulmonary edema, many factors have been involved 1) increased permeability of pulmonary capillaries due to hypoxia (15, 19) 2) movement of fluids to the lungs resulting from an elevated transcapillary gradient due to wide variation of intra alveolar pressure caused by periodic respiratory movements simulating Muller maneuver,[1] and 3) left ventricular dysfunction secondary to hypoxia and hypercapnia (14 18, 24) In our case the elevated left atrial pressure and the reduced ventricular contractility seen in the angiocardiography suggest that left ventricular dysfunction may partially contribute to the genesis of the pulmonary edema The fall in pulmonary artery pressure after endotracheal intubation and the reversibility of disease following surgery emphasizes the significance of a functional factor i e airway obstruction in the genesis of the syndrome

Therapy includes antibiotics for a superimposed respiratory infection digitalis diuretics sitting position of the patient (the supine position exaggerates the obstruction) and tracheal intubation Oxygen administration must be carefully monitored because of the danger of apnea by withdrawal of hypoxic stimulus to the respiratory center Radiation therapy has been suggested (24) as an alternate to the tracheal intubation during the first 2–3 days when the obstruction is critical

The improvement of the patient after hydrocortisone administration indicates that the use of this agent during the first 2–3 crucial days may be helpful This therapeutic modality has been successfully used in the reduction of respiratory obstructions when hypertrophic tonsils and adenoids are combined with upper respiratory infections as in cases of acute mononucleosis (12) Steroids having an anti inflammatory broncholytic and antilympholytic action are a more convenient therapy compared with that of radiation which may damage the thyroid gland The prognosis after early relief of the obstructed airway is usually excellent and the electrocardiographic changes subside within 3–6 months following surgery

[1] Muller maneuver Inspiratory effort with closed nose and mouth

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REFERENCES

1 Ainger L E Large tonsils and adenoids in small children with cor pulmonale *Br Heart J 30* 356 1968
2 Bergofsky E H Lehr D E & Fishman A P The effect of changes in hydrogen ion concentration on the pulmonary circulation *J Clin Invest 41* 1497 1962
3 Bland J W Edwards F K & Brinsfield D Pulmonary hypertension and congestive heart failure in children with chronic upper airway obstruction *Am J Cardiol 23* 830 1969
4 Cayler G G Johnson E E Lewis B E Kortzeborn J D Jordan J & Fricker G A Heart failure due to enlarged tonsils and adenoids *Am J Dis Child 118* 708 1969
5 Cherniack R M & Snidal D P The effect of obstruction to breathing on the ventilatory response to CO_2 *J Clin Invest 35* 1286 1956
6 Cox M A Schiebler G L Taylor W J Wheat M R Jr & Krovetz L J Reversible pulmonary hypertension in a child with respiratory obstruction and cor pulmonale *J Paediatr 67* 192 1965
7 Cronje R E Human G P & Simson I W Hypoxaemic pulmonary hypertension in children *South Afr Med J 40* 2 1966
8 Freeman W J Adenoid hypertrophy cyanosis and cor pulmonale in children with congenital heart disease *Laryngoscope 83* 238 1973
9 Gerald B & Dungan W T Cor pulmonale and pulmonary edema in children secondary to chronic upper airway obstruction *Radiology 90* 679 1968
10 Gresham E L & Armstrong J C Cardiac failure from tonsilar enlargement A reminder *Clin Pediatr 10* 236 1971
11 Grover R F Vogel J H K Averill K H & Blount S G Jr Pulmonary hypertension Individual and species variability relative to vascular reactivity *Am Heart J 66* 1 1963

12 Gutgesell H Jr Acute airway obstruction in infectious mononu leosis *Pediatrics 47* 141 1971
13 Harvey R M & Ferrer M I A clinical consideration of cor pulmonale *Circulation 21* 236 1960
14 Hecht H H Kuida H & Tsagaris T J Brisket disease IV Impairment of left ventricular function in a form of cor pulmonale *Trans Assoc Am Physicians 75* 63 1962
15 Hultgren M H Spickard W B Hellnegal K & Honston C S High altitude pulmonary edema *Medcine 40* 89 1961
16 Himmelstein A Harris P Fritts H W Jr & Cournand A Effect of severe unilateral hypoxia on the partition of pulmonary blood flow in man *J Thorac Cardiovasc Surg 36* 369 1958
17 Jeresaty R M Robert P H & Basu S Pierre Robin syndrome cause of respiratory obstruction cor pulmonale and pulmonary edema *Am J Dis Child 117* 710 1969
18 Levy A M Tabakin B S Hanson J S & Narkewicz R M Hypertrophied adenoids causing pulmonary hypertension and severe congestive heart failure *N Engl J Med 277* 506 1967
19 Luke M J Mehrizi A Folger G M Jr & Rowe R D Chronic nasopharyngeal obstruction as a cause of cardiomegaly cor pulmonale and pulmonary edema *Pediatrics 37* 76? 1966
0 Mahazan C M & Lall J C Cor pulmonale due to upper respiratory obstruction by a laryngeal haematoma *Indian Pediatr 7* 524 1970
21 Menashe V D Farrehi C & Miller M Hypoventilation and cor pulmonale due to chronic upper airway obstruction *J Paediatr 67* 198 1965
22 Menon N D High altitude pulmonary edema A clinical study *N Engl J Med 273* 66 1965
23 Mills J N Changes in alveolar carbon dioxide tension by night and during sleep *J Physiol 122* 66 1953
24 Noonan J A Reversible cor pulmonale due to hypertrophied tonsils and adenoids *Am Pediatr Soc Inc* May 6–8 (abstract) p 48 1965
25 — Pulmonary heart disease *Pediatr Clin North Am* (Nov) Paediatric Cardiology p 1?6? 1971
?6 Pilapil V R Day L H & Watson D G Cor pulmonale resulting from chronic nasopharyngeal obstruction *Surg Forum 18* 493 1968
27 Sanchez J Vizcarrondo F & Martinez Pico A Cor pulmonale due to hypertrophic tonsils and adenoids causing cardiac arrhythmias *Bol Asoc Med PR 63* 07 1971
8 Setliff R C Puyau R A & Ward P H Pulmonary hypertension secondary to chronic upper airway obstruction *Laryngoscope 78* 845 1968

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CASE REPORT

METHOTREXATE AND PREDNISOLONE TREATMENT OF A CHILD WITH PSORIATIC ARTHRITIS

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ABSTRACT Bjorksten B and Back O (Department of Paediatrics and Dermatology University Hospital, Umeå, Sweden) Methotrexate and prednisolone treatment of a child with psoriatic arthritis Acta Paediatr Scand, 64 664, 1975 —A case of severe psoriasis and psoriatic arthritis in a child is presented Only one detailed report has been published previously The result of treatment with prednisolone and methotrexate was encouraging

KEY WORDS Psoriasis in childhood arthritis methotrexate

In psoriasis the skin manifestations are sometimes associated with inflammatory joints lesions and psoriatic arthritis is now considered a definite entity (5) During childhood however, the appearance of psoriatic arthritis seems to be rare and it is only recently that a first documented report on such a case was published (1) In the present paper a child more severely affected with psoriatic arthritis is described and some aspects of the therapy are discussed

CASE REPORT

K A a girl born August 16 1964 Her paternal grandmother suffers from psoriasis as does a maternal aunt At the age of 2 months the girl developed circumscribed skin lesions with scaling over her face scalp and back When 7 months old the lesions were diagnosed as psoriatic by a dermatologist During the following years she was affected by moderate psoriasis In the summer of 1970 when 6 years old she developed a generalized psoriasis and was admitted to hospital for treatment She responded well to topical therapy and was discharged one month later

In the summers of 1971 and 1972 there were severe exacerbations preceded by upper respiratory tract infections In February 1973 she again developed a generalized psoriasis a few days after an upper respiratory tract infection and one month later the arthritis ensued She was then readmitted to the hospital and presented with a fever of 39 4°C but with no other signs of infection She had generalized psoriatic skin lesions (Fig 1) and pitting of the nails There was tenderness with swelling of the fingers (Fig 2) wrists feet ankles and knees Treatment with penicillin and salicylic acid was ineffective and then prednisolone in a dose of 60 mg daily was instituted Within a week a pronounced improvement of the skin lesions as well as of the arthritis was observed She also developed a Cushing like picture and the prednisolone dose was

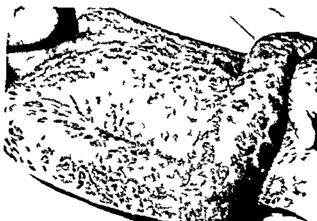


Fig 1 Severe generalized psoriasis on admission in February 1973



Fig 2 Severe psoriasis and arthritis in finger joints and wrists February 1973

gradually reduced However when less than 30 mg pred nisolone daily was given the patient experienced pain and increased swelling of her joints Therefore she was also given 1 75 mg methotrexate thrice weekly at 12 hourly intervals With this combined therapy the pred nisolone dose could gradually be reduced (Fig 3) The patient was discharged and felt well during the summer and autumn of 1973 In December 1973 however she got varicella and the therapy with methotrexate and pred nisolone was immediately withdrawn Within a week she developed widespread psoriatic skin manifestations and swelling of the joints When the infection had subsided the treatment with methotrexate and prednisolone was re sumed (Fig 3) and again she responded well

At the follow up investigation in July 1974 the patient had been treated for 14 months with methotrexate and prednisolone and during this period no side effects of methotrexate had been noticed The only manifestations of her disease were now a faint hyperkeratosis and erythema of the extremities pitting of the nails and swollen metacarpophalangeal joints (Fig 4) but no pain The other previously affected joints appeared normal

Laboratory investigations at regular intervals before and during treatment revealed haemoglobin concentrations of 10 4–15 grams per 100 ml blood white blood cell counts of 5600–21700 and platelet counts of 140000–600000 per mm³ blood normal serum creatinine (0 4–0 7 mg per 100 ml) alkaline phosphatase (6 9–16 Buch & Buch units) and total bilirubin (0 ?–0 3 mg per 100 ml) The serum transaminases S-GOT and S-GPT were normal i e less than 17 units per litre except in January 1974 when she had a severe exacerbation of psoriasis and S-GOT rose to 38 and S-GPT to 58 units per litre

Rheumatoid factor (RF) antinuclear factor (ANF) tests for the presence of LE cells and the antistreptolysin-0 titre were negative on several occasions before and during the period of cytotoxic therapy Quantitative immunoglobulin determinations were within normal ranges for IgG IgM and IgA (11 6–12 3 0 70–0 78 and 1 70–2 25 g per litre serum respectively) Histocompatibility test showed HL A 1 2/8 W15

DISCUSSION

The diagnosis of psoriasis in this girl is established by the typical skin lesions the case history and by the occurrence of psoriasis in close relatives Psoriatic arthritis is a well known entity in adults (5) but rare in childhood The arthritis in this patient is considered to be psoriatic although juvenile rheumatoid arthritis would be a differential diagnosis When the arthritis appeared the patient had

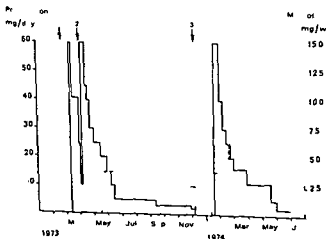


Fig 3 Time scheduled treatment with prednisolone and methotrexathe Arrows indicate respiratory tract infection (1) severe exacerbation of psoriasis (2) and varicella (3)



Fig 4 Minor psoriatic lesions remaining after treatment with prednisolone and methotrexate on admission in July 1974 Cf Fig 1

suffered from psoriasis for more than 8 years and the onset of the arthritis was preceded by a severe attack of psoriasis Repeated tests for RF and ANF were negative This however, occurs frequently in juvenile rheumatoid arthritis (2) The arthritis in this girl was polyarticular and no other clinical features of acute juvenile rheumatoid arthritis were observed Considering the above facts psoriatic arthritis would be the most appropriate diagnosis

Considerable experience of methotrexate treatment of psoriasis in adults has now been collected (3), but no report on treatment with this cytotoxic drug for psoriasis in childhood has yet been published as far as we know Side effects of cytotoxic drugs are common and may be severe Thus the indication for their use must be very strict The recommended dose of methotrexate for the treatment of malignant diseases in children is 20–60 mg per m² body surface once weekly (4) and with this dose side effects are common We used 2–8 mg methotrexate per m² weekly a dose commonly used in psoriatic adults (3) The patient responded well only to a high dose of prednisolone and in an effort to control the disease and to reduce the side effects of corticosteroids a combined treatment with methotrexate and prednisolone was started The patient responded well to this treatment and she has remained well on 7 5 mg methotrexate weekly for more than 3 months after withdrawal of steroids Thus a combination of methotrexate and prednisolone may be of value in the treatment of severe psoriasis in childhood

REFERENCES

1 Angevine C D Pless I B & Jacox R F Psoriatic arthritis in a child *Arthritis Rheum 16* 278 1973
2 Brewer E J *Juvenile rheumatoid arthritis* W B Saunders Co Philadelphia 1970 p 76
3 Roenigk H H Maibach H I & Weinstein G P Methotrexate therapy for psoriasis *Arch Dermatol 108* 35 1973
4 Sutow W W Vietti T J & Fernbach J *Clinical pediatric oncology* C V Mosby Co Saint Louis 1973 p 104
5 Wright V & Moll J M H Psoriatic arthritis *Bull Rheum Dis 21* 627–632 1971

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CASE REPORT

DISSEMINATED INTRAVASCULAR COAGULATION AND CONGENITAL NEUROBLASTOMA

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S WILHELMSSON AND L ÅSTRÖM

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ABSTRACT Faxelius G Teger Nilsson A-C Wilhelmsson S and Åstrom L (Department of Pediatrics Department of Clinical Chemistry and Department of Blood Coagulation Research Karolinska sjukhuset Stockholm Sweden) Disseminated intravascular coagulation and congenital neuroblastoma Acta Paediatr Scand 64 667 1975 A newborn infant with congenital neuroblastoma complicated by disseminated intravascular coagulation is described At birth the infant showed liver and spleen enlargement and shortly thereafter malignant cells were found in the bone marrow On the fifth day of life the infant started to bleed and coabulation analysis indicated disseminated intravascular coagulation Heparin therapy corrected the coagulation anomaly and irradiation and chemotherapy temporarily improved the general condition of the infant The infant finally succumbed from its primary neoplastic disease

KEY WORDS Infant congenital neuroblastoma disseminated intravascular coagulation heparin therapy

Disseminated intravascular coagulation in the neonatal period has been described in several diseases (4 9 11) mainly respiratory disorders (3 2 12 13) viral infections (18) septicemia (1) and rhesus immunization (5) Intravascular coagulation in the course of neuroblastoma has only been described in one infant previously (14) We therefore found it of interest to report an infant with congenital neuroblastoma complicated with disseminated intravascular coagulation

METHODS

Blood sampling During the first 3 days of life blood specimens were drawn from an umbilical venous catheter Thereafter they were collected by heel puncture or from a

Total platelets were determined according to Kristenson (10)

Activated partial thromboplastin time (APTT) was determined with Platelin Plus Activator (American Optical Co) using a micromodification of the manufacturer s directions

Table 1 *Coagulation analyses at the fifth day of life*

	Patient s values	Reference values*
Platelets/µl	55 000	120 000–460 000
APTT s	303	36–86
Factor V %	48	76–124
Prothrombin %	26	76–85
Thrombotest %	31	27–74
Fibrinogen g/100 ml	0 09	0 22–0 56
FDP mg/100 ml	2 5	<0 5

* Mean ±2 S D for normal newborns

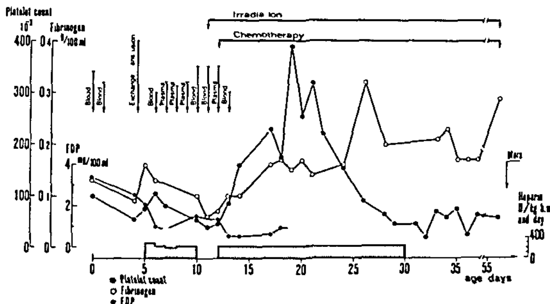


Fig 1 Platelet count (●—●) fibrinogen (O—O) and fibrinogen – fibrin degradation products (*—*) of the infant Therapy with blood products heparin irradiation and cytostatics is indicated

Factor V was determined according to Wolf (19)

Prothrombin was determined according to Norén (17)

Prothrombin complex (Factors II (IX) X VII) was determined with Thrombotest The values were corrected to a haematocrit of 45%

Fibrinogen was determined according to de Vreker et al (7)

Fibrinogen – fibrin degradation products (FDP) in serum were determined according to Nihlén (16) Antifibrinogen (Dakopatts) was used as antibody and diluted normal plasma as standard

CASE REPORT

A female infant was born at term after an uneventful pregnancy Delivery was uncomplicated Apgar score normal and body wt 3060 g Physical examination immediately after birth showed a large abdomen with enlarged liver and spleen Several purpuric spots were distributed over the body

Pertinent laboratory findings were Haemoglobin 13 5 g/100 ml haematocrit 40–34% during the first hours Thrombotest 43% platelet count 100 000/μl fibrinogen 0 13 g/100 ml white cell count 43 000/μl bilirubin 1 6 mg/100 ml with no further increase later in the course There was no clinical or laboratory evidence of blood group incompatibility septicemia or viral disease X ray of the abdomen showed the liver and spleen to be enlarged Portography gave no evidence of thrombosis although there was no contrast filling of the vessels of the lower part of the right liver lobe

Over the next days the size of the liver increased On the third day of life neuroblastoma was suspected as bone marrow showed invasion of malignant cells The diagnosis was later confirmed by highly increased levels of homovanillic and mandelic acid in urine On the fifth day of life the infant started to bleed from the umbilical tract and showed prolonged bleeding from heelprick sites Coagulation analyses revealed abnormal values (Table I) An exchange transfusion with heparinized blood was performed and heparin therapy was started with a dosage of 300–240 U/kg body wt and day given i v at 4-hour intervals After exchange transfusion and heparinization the bleeding ceased and the coagulation analyses slowly normalized (Fig 1)

Irradiation and chemotherapy was started on the eleventh day of life Radiation treatment ^{60}Co apparatus with a large frontal field covering the whole abdomen Tissue dose in the middle of the abdomen was calculated to 1 100 T within 43 days Chemotherapy Cyclophosphamide 10 mg/kg body wt i v every other week and Vincristine 0 05 g/kg body wt i v every other week

The condition of the infant improved after the first weeks of irradiation and chemotherapy Subsequently increasing liver size and abdominal growth and severe breathing difficulties were noted At 2 months of age an explorative laparotomy was performed on vital indication At operation an enormously enlarged liver was found However no primary tumour was located The infant expired shortly after operation

At autopsy a neuroblastoma was found in the right adrenal gland (weight 28 g versus the normal 4–6 g in this age group) with massive metastasis to the liver (weight 930 g versus the normal 140 g) A large subcapsular hematoma was found in the liver a problable immediate cause of death

DISCUSSION

The diagnosis of disseminated intravascular coagulation was established on the fifth day of life but it might have been present at birth Diagnosis was based on the clinical picture as

well as on typical depletions of coagulation factors The low platelet count was not necessarily due to intravascular coagulation but could equally well have been the result of malignant infiltration of the bone marrow The course of events however with increasing platelet count during heparin therapy showed that the bone marrow was capable of platelet production at least in the early stage of the disease The low concentration of plasma coagulation factors could also be the result of an impaired liver function Essentially normal values of prothrombin thrombotest as well as normal bilirubin concentration argue against serious damage to liver function Subacute disseminated intravascular coagulation was therefore the most reasonable explanation and it was believed to be due to presence in the blood of thromboplastic material from the malignant cells a phenomenon which is seen in several other malignant diseases with metastasis (15)

The infant received an exchange transfusion with heparinized fresh blood followed by repeated transfusions and heparin therapy On the eleventh day of life an attempt was made to discontinue the heparin therapy However platelets and fibrinogen decreased in spite of continuous blood transfusions The heparin therapy was therefore restarted and given for 30 days No bleeding and no other side reactions were noted At the end of the first month of the life platelet count decreased in spite of heparin therapy but fibrinogen concentration remained essentially unchanged The thrombocytopenia in the final stage of the disease was probably essentially due to bone marrow damage

The child fulfilled the criteria for stage IV S of neuroblastoma according to Evans et al (8) and would have an expected 2 year survival of 90% (6) In this case however it was not possible to save the life of the infant who eventually succumbed to the tumour The relatively good prognosis made all efforts to diagnose and treat the coagulation anomaly worthwhile Although intravascular coagulation seems to be a rare complication it should be given consideration in cases of neuroblastoma in infancy

ACKNOWLEDGMENTS

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REFERENCES

1 Alebouyeh M Remien A & Marget M Incidence of disseminated intravascular coagulation in the course of septicemia in newborn infants *Z Kinderheilkd 109* 3 6 1971
2 Altstatt L B Dennis L H Sundell H Malan A Harrison V Hedvall G Eichelberger J Fogel B & Stahlman M Disseminated intravascular coagulation and hyaline membrane disease *Biol Neonate 19* 227 1971
3 Bleyl U Busing C & Krempien B Pulmonale hyaline Membranen und perinataler Kreislaufschock *Virschows Arch (Pathol Anat) 348* 187 1969
4 Boyd J Disseminated fibrin thrombo-embolism in stillbirths a histological picture similar to the form of maternal hypofibrinogenemia *J Obstet Gynaecol Br Commonw 73* 629 1966
5 Chessels J M & Wigglesworth J S Haemostatic failure in babies with rhesus isoimmunoisation *Arch Dis Child 46* 138 1971
6 d Angio G Evans A & Koop E Special pattern of widespread neuroblastoma with a favourable prognosis *Lancet I* 1046 1971
7 de Vreker R A Vermylen C & Verstraete M A rapid enzymatic method for assay of fibrinogen fibrin polymerization time (FPT test) *Clin Chim Acta 8* 418 1963
8 Evans A d Angio G & Randolph J A proposed staging for children with neuroblastoma *Cancer 27* 374 1971
9 Hathaway W E Mull M M & Pechet G S Disseminated intravascular coagulation in the newborn *Pediatrics 43* 233 1969
10 Kristenson A A new method for the direct counting of the so-called blood platelets in man *Acta Med Scand 57* 301 1922
11 Leissring J C & Vorlichy L N Disseminated intravascular coagulation in a neonate *Am J Dis Child 115* 100 1968
12 Markarian M Githens J H Rosenblut E Fernandez F Jackson J J Bannon A E Lindley A Lubchenco L O & Martorell R Hypercoagulability in premature infants with specia reference to the respiratory distress syndrome an hemorrhage I Coagulation studies *Biol Neonate 17* 84 1971
13 Markarian M Lubchenco L O Rosenblut E Fernandez F Lang D Jackson J J Bannor

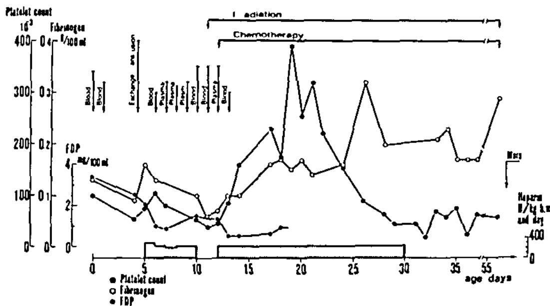


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CASE REPORT

PROXIMAL RENAL TUBULAR ACIDOSIS IN TETRALOGY OF FALLOT

J RODRIGUEZ SORIANO A VALLO M CHOUZA and G CASTILLO

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ABSTRACT Rodriguez Soriano J Vallo A Chouza M and Castillo G (Department of Paediatrics Hospital Infantil de la Seguridad Social Bilbao Spain) Proximal renal tubular acidosis in tetralogy of Fallot. Acta Paediatr Scand 64 671 1975.—A 9 year-old girl presented with tetralogy of Fallot and moderate metabolic acidosis Despite a Blalock s fistula there was evidence of chronic hypoxia with cyanosis clubbing of fingers and toes and very elevated blood hematocrit values Renal acidification and bicarbonate titration demonstrated the existence of proximal renal tubular acidosis renal bicarbonate threshold was low (18 mmoles/l) and normal urinary acidification was present at subthreshold serum bicarbonate levels Following corrective heart surgery blood acid–base values and renal reabsorption of bicarbonate became normal A causal relationship between extracellular fluid volume expansion dependent on the high hematocrit and proximal renal tubular acidosis is suggested

KEY WORDS Tetralogy of Fallot proximal renal tubular acidosis metabolic acidosis acid–base balance

Metabolic acidosis is a common event in association with congenital cyanotic heart disease (7) and an increased lactacidemia secondary to chronic hypoxemia is generally implicated in its genesis (8 12) Recent findings however indicate that non lactic acidosis may also be present due to an associated defect in the renal reabsorption of bicarbonate (1 2) The present report demonstrates this abnormality in a patient with tetralogy of Fallot and its complete disappearance following total corrective heart surgery

CASE REPORT

A girl 9 $^{1}/_{12}$ years old was admitted to hospital because of generalized convulsions She had been diagnosed previously as a case of Fallot s tetralogy and a palliative shunt operation according to Blalock Taussig had been performed at 5 years of age Seizures have recurred intermittently since 18 months of age On physical examination she was comatous and outstanding findings were moderate growth retardation (height 1 0 cm weight 22 5 kg) generalized cyanosis and marked clubbing of fingers and toes The clinical diagnosis of Fallot s tetralogy along with the patency of the Blalock s fistula was subsequently confirmed by cardiac catheterization and angiocardiography

At the time of admission blood hematocrit was elevated (73 %) and a moderate metabolic acidosis was present (Fig. 1) Following substitution of 700 *ml* of seroalbumin for the same amount of blood a marked improvement took place and the level of consciousness became normal Hematocrit decreased to 60 % but the metabolic acidosis persisted Other blood laboratory data were normal sodium 135 mEq/l potassium 4 8 mEq/l osmolality 282 mOsm/l urea 37 mg/100 ml calcium 9 7 mg/100 ml phosphorus 4 2 mg/100 ml Neither protein nor reducing substances were present in the urine Early morning urinary osmolality attained 844 mOsm/l Tubular reabsorption of phosphorus (TRP) was 88 %

Administration of 60 *mEq/day of sodium bicarbonate* was followed by complete normalization of acid–base data and that therapy was maintained until the time of cardiac surgery 3 months later Withdrawal of therapy during 24

A E Lindley A Githens J H & Martorell R Hypercoagulability in premature infants with special reference to the respiratory distress syndrome and hemorrhage II The effect of heparin *Biol Neonate 17* 98 1971
14 McMillan C W & Gaudry Jr C L Coagulation defects and metastatic neuroblastoma *J Pediatr 72* 347 1968
15 Merskey C Defibrination syndrom *In* R Biggs (ed) *Human blood coagulation haemostatis and thrombosis* Blackwell Scientific Publications Oxford 1972 p 444
16 Nihlén J E Separation and estimation of split products of fibrinogen and fibrin in human serum *Thromb Diath Haemorrh 18* 487 1967
17 Noren I Specific assay of prothrombin A method using a freeze-dried reagent of intrinsic coagulation factors *Scand J Clin Lab Invest* 25 47 1970
18 Shershaw L W Ekert H Swanson U L Wright H T & Gilchist B Intravascular coagulation in generalized herpes simplex infection of the newborn *Acta Paediatr Scand 58* 535 1969
19 Wolf P A modification of routine laboratory use of Stefanini s method of estimating factor V activity in human oxalated plasma *J Clin Pathol 6* 34 1953

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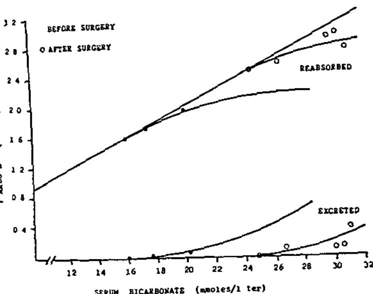


Fig 2 Reabsorption and excretion of filtered bicarbonate during continuous bicarbonate infusion Time and conditions at the time of titration studies are described in Table 1 and in Fig 1

bicarbonate Renal bicarbonate threshold was low and a normal urinary acidification was achieved at subthreshold bicarbonate levels thus defining the proximal type of renal tubular acidosis (10 11) No abnormalities were found in glomerular or other tubular functions A defect in tubular reabsorption of bicarbonate in patients with tetralogy of Fallot has already been suspected (1 2) but no studies are available showing its complete disappearance after corrective heart surgery as was demonstrated in our patient thus demonstrating that the renal abnormality is directly related to the cardiac lesion

We can only speculate about the nature of the renal disorder Aperia et al (1) have emphasized the possible role of low arterial P_{CO_2} values due to hyperventilation found in patients with Fallot s tetralogy (3 4) as a cause of a secondary depression of renal reabsorption of bicarbonate In our patient P_{CO_2} levels were not constantly decreased and no obvious relationship was evident between them and the degree of metabolic acidosis Aperia et al (1) have also shown that the natriuretic response to an oral sodium load was lower in patients with tetralogy of Fallot than in normal subjects and that the urinary sodium excretion correlated inversely with the filtration fraction which was markedly elevated in the cardiac

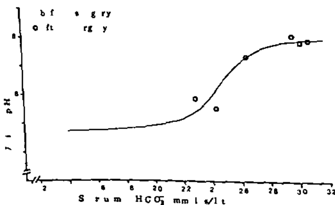


Fig 3 Relationship of urinary pH to concentration of bicarbonate in serum The curve represents the average relationship found in normal children (6)

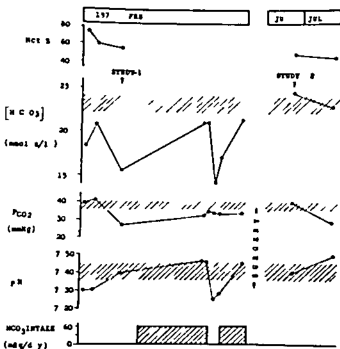


Fig 1 Clinical course Biochemical features as influenced by alkali therapy and corrective heart surgery

hours when cardiac catheterization was performed was immediately followed by reappearance of metabolic acidosis A complete correction of Fallot s tetralogy with ligation of the previous fistula was performed when the patient was $1^{1}/_{12}$ years old Postoperatively cyanosis was absent and a good general condition was maintained with digitalis therapy Follow up studies of acid–base balance have been repeatedly normal and no alkali treatment has been required any longer

Special studies

Assessment of tubular reabsorption of bicarbonate was performed according to the procedure described by Edelmann et al (5) Special care was taken to minimize extracellular fluid volume expansion Glomerular filtration rate (GFR) was estimated by endogenous creatinine clearance Laboratory determinations were carried out as described by one of us (10)

RESULTS

The study of tubular reabsorption of bicarbonate was performed twice before and 2 months after complete correction of Fallot s tetralogy Pertinent clinical and physiological data at the time of the studies are given in Table 1 Results are set out in Fig 2 Rates of reabsorption and excretion of bicarbonate are related to 100 ml GF and plotted against serum bicarbonate concentration Before surgery a low renal bicarbonate threshold of 18 mmoles/litre established the diagnosis of proximal renal tubular acidosis After surgery a normal threshold of 25 mmoles/litre was reached

In Fig 3 urine pH is plotted as a function of bicarbonate concentration in serum both in the condition of induced metabolic acidosis and in alkalosis The curve represents the average relationship found in normal children (6) Before surgery the points are skewed to the left of the normal line indicating the lowering of the renal bicarbonate threshold a normal urinary acidification—with a minimal urinary pH of 4 90—is present but this is only accomplished at serum levels below 16 mmoles/litre After surgery the points follow the normal line and comparable levels of urinary pH are achieved at higher levels of serum bicarbonate

DISCUSSION

This 9 year old girl presented with moderate metabolic acidosis in association with congenital heart disease due to Fallot s tetralogy Despite a Blalock s fistula there was evidence of chronic hypoxia with cyanosis clubbing of fingers and toes and very elevated blood hematocrit values Although serum levels of lactic acid were not determined and some elevation cannot be excluded there is no doubt that the metabolic acidosis was caused mainly by a defect in the renal reabsorption of

Table 1 *Clinical and physiological data at the time of the bicarbonate titration studies*

Study	Age (years)	Blood pressure (mmHg)	Hematocrit (%)	Pa_{O_2} (mmHg)	Serum potassium (mEq/l)	Creatinine clearance (ml/min/1 73 m²)
1	$9^{11}/_{12}$	115/75	55	62	4 8	108
2	$10^{3}/_{12}$	120/80	46	84	4 4	99

BOOK REVIEWS

J L Melnick (ed) *Progress in medical virology* vols 15 and 16 S Karger Basel Munchen Paris London New York Sydney 1973 Vol 15 384 pp illus US $31 95 Vol 16 363 pp illus US $30 40

Progress in Medical Virology has proved to be a series of utmost interest to everyone working in virology and epidemiology The first volumes of the series are now out of print and rather than reprinting previous volumes the editor has chosen to include in future volumes rewritten and up-dated reviews on selected topics Most of the authors to rewritten arti les are identical with those con tributing a decade or more ago In volume 15 of 1973 8 of 11 articles are up-dating of previously published reviews and in volume 16 3 out of 9 are rewritten

The field of knowledge in prenatal and perinatal infec tions has expanded much during the last 10–15 years and new information on the role of viruses in congenital malformations is rapidly accumulating particularly regard ing rubella and cytomegaloviruses *Blattner Williamson & Heys* have in volume 15 up-dated their more than 10-year-old article on this subject and they present an in formative survey comprising many of the still increasing group of agents known to cause fetal damage It is re cognized today that congenital cytomegalovirus infection constitutes a health problem which seems to exceed that of rubella in terms of fetal damage and human tragedy This area might perhaps have deserved greater attention but a substantial part of the new information on intra uterine cytomegalovirus infections has accumulated since 1970 and the list of references in this article does not comprise many publications of a later date *Plummer* has in a special chapter given an excellent review of the progress made in the cytomegalovirus field since 1958 when Margaret G Smith presented her survey entitled The alivary gland viruses of man and animals Plummer ummarizes the effects of cytomegaloviruses on their iosts and their taxonomic relationship to the other herpes iruses His survey includes a very comprehensive and up o date list of references

Volume 15 contains plenty of interest for those in erested in infections of the newborn child *Gear & Measroch* have up-dated Gear s 1958 review on coxsackie virus infections of the newborn They survey outbreaks of coxsackie virus type A and B and outline preventive measures The authors suggest that the future epidemiology of coxsackie virus may develop like that of polio This means that improved standards of living will lead to postponements of infection into higher age groups But they also expect an increased number of babies to be born without the protection of maternal antibodies to cox sackie viruses Even the following chapter deals with coxsackie virus infections *Lerner & Wilson* survey ad vances that have taken place since they wrote their ar ticle on virus myocardiopathy 8 years ago The authors critically review more than 100 selected reports of human and experimental animal infections Coxsackie virus group B infections may be associated with acute myopericardi tis valvulitis chronic myocardiopathy constrictive peri carditis obstructions of vena cava endocardial fibro-elastosis and congenital malformation of the heart Coxsackie viruses group A and echoviruses are also suspected to cause some of these disorders and it is sug gested that cases of rheumatic heart disease and glomerulonephritis might also be due to enterovirus infec tions

In 1959 in volume ? of this series *Ward* review viruses of the respiratory tract Data on these agents has proliferated rapidly calling for a revision of his article and Ward has now given an informative current survey on myxo- rhino-adeno and corona viruses A topic previously not dis cussed but of great current interest is the report by *Foege & Eddins* on mass vaccination programs in de veloping countries They describe recently acquired ex perience in such activities and discuss areas to be con sidered in new immunization projects

Those working in diagnostic virology will find particu larly useful information in the exquisite review by *Schmidt & Lennette* on advances in the serodiagnosis of viral infec tions This is an up-dating and extension of the article written by the same authors in 1961 Of great current interest is also *Cockburn s* chapter on the WHO pro-gramme in medical virology and the developments achieved in the organization s virologic activities since Cockburn wrote his previous report on the same subject in 1966

The three last chapters of volume 15 deal with topics in basic virology *Higashi*—one of the pioneers in the electron microscopy of viruses in thin sections of cells grown in culture—has up-dated and extended his chapter of 1959 Such studies have contributed to the understand ing of the structure and morphogenesis of virus particles *Barrett Calendar G bbs Goldstein Lindqvist & Six* re view the state of knowledge in area of helper-dependent bacteriophage and discuss the potential implications for animal virology and oncology

The 16th volume of Progress in Medical Virology also appeared in 1973 This volume contains two outstanding articles focusing upon viral oncogenesis *Casto & DiPaolo* review studies on the interactions of chemicals viruses and radiation and *McAlister* gives an informative survey on viruses in human carcinogenesis Similarly the articl by *Horzinek* on the structure of togaviruses and the excel lent survey of *Levine* on the effects of irradiation on th responses of animal cells to virus deal particularly wit basic virology *Boulter & Appleyard* discuss difference between extra and intracellular forms of poxvirus and th implications of this phenomenon with particular emphas

patients They suggested that the diminished urinary sodium excretion was probably caused by the high hematocrit and intrarenal physical forces since no hyperaldosteronism was present According to these studies it can be assumed that the extracellular fluid volume is probably expanded in some subjects with high hematocrit due to cyanotic heart disease through the increased tendency to sodium retention When this situation occurs it could lead directly to a depression of renal reabsorption of bicarbonate since there is strong evidence that this reabsorption is dependent on variations of extracellular fluid volume (9)

It appears that patients with cyanotic heart disease may benefit from sodium retention by increasing their plasma volume and thereby preventing the hematocrit from reaching high levels (1) At a certain level of volume expansion however regulation of extracellular volume might become preferential and sodium bicarbonate escape into the urine The benefit of sodium retention is lost and a further elevation of hematocrit may be followed by neurological complications as was the case in our patient

If the above hypothesis is correct it may have a direct implication on the management of the metabolic acidosis It should be treated by measures tending to increase the plasma volume such as the administration of sodium bicarbonate It is known that hydrochlorothiazide may be useful in the treatment of proximal renal tubular acidosis through the increased tubular reabsorption of bicarbonate induced by the extracellular fluid volume contraction caused by the sodium loss (9) In patients with cyanotic heart disease and associated metabolic acidosis this therapy might be potentially dangerous since it could be followed by an acute elevation of hematocrit and a threatening neurological complication

REFERENCES

1 Aperia A Bjarke B Broberger O & Thoren C Renal function in Fallot s tetralogy *Acta Paediatr Scand 63* 398 1974

2 Callis L Castelló F & Vallo A Acidosis tubular proximal en las cardiopatias congenitas cianosantes *Proceedings of the 2nd International Symposium of Pediatric Nephrology* Paris August 25–28 1971 p 197

3 Campbell J M H Hunt G H & Poulton E P On examination of blood gases and respiration in disease with reference of the cause of breathlessness and cyanosis *J Path Bact 26* 234 1923

4 Davies H & Gazetopoulos N Dyspnea in cyanotic congenital heart disease *Br Heart J 27* 28 1965

5 Edelmann C M Jr Rodriguez Soriano J Boichis H Gruskin A B & Acosta M I Renal bicarbonate reabsorption and hydrogen ion excretion in normal infants *J Clin Invest 46* 1309 1967

6 Edelmann C M Jr Boichis H Rodriguez Soriano J & Stark H The renal response of children to acute ammonium chloride acidosis *Pediatr Res 1* 452 1967

7 Folger G M Acidemia of cardiogenic origin in young infants with cyanotic congenital heart abnormalities *Clin Pediatr 11* 573 1972

8 Gootman N L Scarpelli E M & Rudolph A M Metabolic acidosis in children with severe congenital heart disease *Pediatrics 31* 251 1963

9 Martinez Maldonado M Eknoyan G & Suki W N Diuretics in nonedematous states Physiological basis for their clinical use *Arch Intern Med 131* 797 1973

10 Rodriguez Soriano J Boichis H Stark H & Edelmann C M Jr Proximal renal tubular acidosis A defect in bicarbonate reabsorption with normal urinary acidification *Pediatr Res 1* 81 1967

11 Rodriguez Soriano J The renal regulation of acid–base balance and the disturbances noted in renal tubular acidosis *Pediatr Clin N Am 18* 529 1971

12 Rudolph A M Metabolic and hemodynamic adjustments to hypoxia in infancy *Bull N Y Acad Med 42* 987 1966

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STANDARDS AND INDICATIONS FOR INDUSTRIALLY PRODUCED INFANT FORMULAS

Some Principles

BERTIL LINDQUIST

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In December 1973 an article was published in the Medical Tribune written by the Medical Tribune science editor entitled Baby feeds —are the big companies doing enough? Apart from the content of the article the question seems important and reflects what many people in general including politicians journalists etc are thinking of today To this question can be added another one are the pediatricians especially pediatricians working in the field of nutrition doing enough? Certainly a lot of important research in infant nutrition has been done—and is still being done—by industrial scientists in addition to the research done by university scientists As however industrially produced infant formulas are available to more and more babies in the world the responsibility of baby food manufacturers as well as of the pediatricians is really great

Codex Alimentarius Commission has designated a special committee[1] to work out some basic rules for infant formulas especially with regard to composition of single nutrients and to quality factors There remain however certain problems with respect to infant feeding with industrially produced infant formulas where it probably will be difficult to arrive to any (official) statements by this committee Some of the problems are of practical importance and involve terminology and classification of infant formulas Other problems concern food ingredients which may be used in the production of such formulas In addition the ethical aspects of sales promotion—a field of growing interest during recent years—will be commented upon in this article

Terminology and Classification of Infant Formulas

General terminology Terminology of the product is a question of practical importance especially with respect to comparison between different products on a national as well as an international level Still today—in spite of the work by the Codex Committee—many names are used for products designed for bottle feeding of infants such as infant milks infant formulas milk dried preparations milk mixtures breast or mother s milk substitutes etc etc Other more specific terms are humanized infant formulas adapted milks etc

It would according to my opinion be of great value (for the pediatricians for the producers as well as for the consumers) if one could agree upon a term that is common for all kinds of bottle feeding products (primarily liquid or after reconstitution) and that could be trans

Presented at a meeting on Infant Nutrition in Trieste June 28 1974

[1] Codex committee on foods for special dietary uses

on its significance for immunity to pox virus infections and in the development and assay of vaccines

Horstmann has contributed an article of utmost current clinical interest She describes the available methods for serological surveillance of vaccinated populations These are used to ensure that supposedly well-immunized populations really are immune to determine that sufficient herd immunity has been established and to investigate the persistence of vaccine induced antibodies *Wenner* has brought together in a most valuable review clinical epidemiological and biological aspects of virus infections associated with eruptive fevers Special attention is given to virus diseases which have not been recently reviewed and to informations gained in the last decade since the earlier review in this series by Wenner & Lou A very comprehensive and up to date bibliography subgrouped in accordance with the viruses discussed has been included

To the present writer working in clinical virology and with experience from the virological diagnostic activity in Sweden the article of *Bradstreet Pereira & Pollock* is of special interest and it might serve as a good example in many countries The authors up-date their 10 year-old publications in this series on the organization of a national virological diagnostic service They describe development organization and current activities of the public health laboratory service a national service covering England and Wales From the Central Public Health Laboratory at Colindale (London)—a group of reference and special laboratories—the scheme spreads to ten regional laboratories located at university centres and to more than 50 area laboratories situated throughout the regions served The features of this virological service include individual and group research training of medical and technical staff and students accumulation and analyses of weekly reports thus providing an early warning system concerning viral infections in the country All laboratories are supplied with reagents from the Central Standards Laboratory Service The laboratories participate in virologic investigations set up by the Epidemiological Reference Laboratory There is no doubt that this organization has to a great extent made possible many of the outstanding collaborative studies which collect valuable information of benefit not only to Britain but also to the whole world

Like previous books of this series the 15th and 16th volumes conclude with a chapter by the *Editor* on progress in classification and nomenclature of animal viruses In volume 16 *Melnick* states that the time seems to have arrived for the International Committee on Nomenclature of Viruses to become the International Committee on Taxonomy of Viruses The *Editor* reports on the separation of RNA and DNA-containing viruses into major groups on the basis of physical and chemical properties and he includes a tabulation of virus groups in terms of the polymerases which they contain or induce in the infected cell

Gun Carlstrom

M F Woodburn *Social implications of spina bifida—A study in S E Scotland* Scottish Spina Bifida Association Eastern Branch 14 Watson Crescent Edinburgh EH11 1HE 1974 £2 90

This volume is based on detailed interviews with families having a child with spina bifida 74 of them suffering from myelomeningocele It was decided to include all children older than eighteen months at the time of the interview and almost all known cases in the area were traced The medical and social situation of the families has been documented in a great number of tables and practically all aspects of the frequently immense problems have been illustrated All the difficult problems with bladder and bowel incontinence the need for various appliances the threat of shunt dysfunction the sometimes disturbed relations within the family the limited mobility of the child which sometimes leads to a social isolation of the family all these circumstances add up to a heavy burden for the family to cope with It is beyond doubt that the spina bifida patient and his family needs every possible medical and social support both from a specialized spina bifida team and from local medical and social services Everyone caring for a spina bifida child whether part of a spina bifida team or not could benefit from learning from the experiences collected in this volume It is of special interest to learn that many of the suggestions for treatment are difficult to carry through in practice such as the use of heavy orthopaedic braces difficult to put on and difficult to carry The problems of urinary incontinence are not eradicated in all patients in which a cutaneous ureteroileostomy has been carried out

This study of the social implications of spina bifida is of a great value in the current discussion of selection of the patients for the primary operation of myelomeningocele The author can be recommended to publish a more concentrated report of this interesting investigation in order to make it more easy to read and more easily available

B Hellstrom

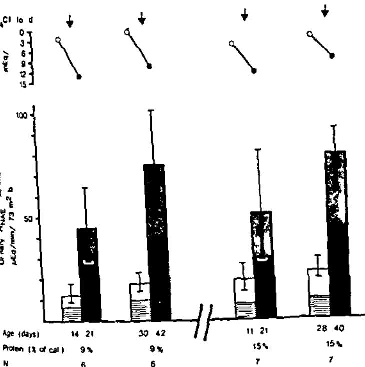


Fig 2 Influence of protein intake on plasma deficit and urinary H excretion before and during induced acidosis in preterm infants at 1–3 weeks and 4–6 weeks respectively of postnatal life *Symbols* Preloading and maximum plasma base deficit (mean) ○—● urinary H NAE (mean and range) I I H TA (mean) and H NH4 (mean) *Note* the low hydrogen excretion capacity during the first 2–3 weeks of life and the wide range of this capacity notable also at 4–6 weeks of life From Svenningsen & Lindquist, 1974

Definition of adapted formulas Today there is no internationally accepted definition of adapted (or humanized) formulas The meaning of such a term may vary from country to country and from one manufacturer to another It would therefore be of great value if from an international point of view one could agree upon a definition of the term adapted formula i e which requirements should be fullfilled for an infant formula to be called adapted

Generally speaking an adapted formula should be similar in composition and appearance to human milk Such a formula should thus not be permitted to contain starch (or flour) Acidification by addition of dl acid should not be permitted and one wonders whether acidification should be permitted t all

The adapted infant formula should fullfill certain requirements with respect to the concentration of different nutrients (protein lipids carbohydrates minerals) and furthermore certain quality criteria should be fullfilled Last year the Committee on Nutrition of the German Pediatric Society has published its definition of an adapted formula (3) and this seems in most respects to be a sound base for further discussions of this matter

Comments on Some Ingredients in Infant Formulas

As mentioned before the Codex Committee has stated in the present draft that infant formulas should meet the nutritional requirements of normal infants Consequently the draft contains recommendations about the content (minimum and sometimes maximum level) for the most important single nutrients and also certain quality criteria These considerations seems to have been mainly accepted by the pediatricians and therefore they don t need to be discussed here

However certain ingredients in infant formulas to cover the requirements of various single nutrients are still the subject of controversial interest The use of such ingredients needs to be further discussed including development of sound recommendations

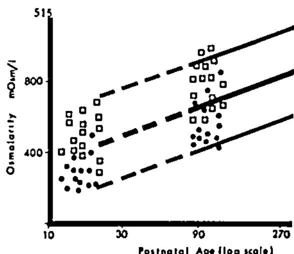


Fig 1 Renal concentration capacity in early infancy *Symbols* □ Fullterm ● Preterm infants Regression lines (Mean ±2 S D) according to Winberg (*Acta Paediatr Scand 48* 318 1959) From Svenningsen & Lindquist 1971

lated into most languages For this purpose the term infant formula seems to be the most adequate (in German Formula Diat fur Sauglinge in French Formule pour Nourrisant in Swedish Formulakost etc) [1]

There exist in many countries today two types of infant formulas so called starting milks and follow up milks or better expressed starting formulas and follow up formulas Starting formulas (Anfangsnahrung) are intended to be used during the first 4–6 months of life Consequently they should completely cover the nutritional requirements at this age as they are the only or main food of the infant Follow up formulas (Dauernahrung) are used for the following months of infancy and they are intended to be used together with increasing amounts of different semisolid and solid foods at this age—second part of infancy—ordinary cow s milk replaces more and more (varying in different countries) the industrially produced formulas As the follow up formula is just a part of a mixed feeding scheme, the composition of follow up formulas may vary according to feeding habits in different countries and in different parts of the world [2] The manufacturers should be asked to indicate for each product which category applies

The above mentioned proposals about classification of infant formulas refer only to conventional infant formulas (designed for healthy babies) and not to so-called therapeutic formulas designed for treating babies with different disorders

The term humanized (adapted) formula The terminology used for that type of starting formula intended to be used especially during the immediate postnatal adaptation period—when different organ functions are less well developed—needs some specific comments It is now well known that within the normal development of renal as well as of other important organ functions, there is a wide biologic variation in the rate of maturation This is illustrated in Fig 1 and Fig 2 showing the renal concentration capacity and the renal acidifying capacity respectively in early life (15 17) It seems thus not appropriate to refer to means when different organ functions are under consideration in relation to the metabolic load of a formula The infants of interest in this connection are those on the *minus* side of the normal range

Starting formulas which are developed for use especially during the immediate postnatal adaptation period are in certain countries called humanized infant formulas in other countries adapted infant formulas There seems to be a general agreement today that the term humanized is not a very good term An infant formula can never be humanized It should therefore be of great value if this term could be abandoned and replaced by the term adapted For the same reasons the term fully adapted should be deleted because a formula can of course never be fully adapted

[1] Cf definition of formulated foods by AMA Council on Foods and Nutrition Formulated foods are mixtures of two or more foodstuffs or ingredients other than seasonings processed or blended together (1)

[2] It may be noted that the 27th World Health assembly has in a resolution on infant nutrition and breast feeding requested the Director General to promote and further support activities related to the preparation and use of weaning foods based on local products (WHA 27/43 23 May 1974)

Adequate acidification of a formula with l acid thus appears to be relatively harmless The main indications for use of such formulas in feeding normal infants are in situations when good hygienic conditions and adequate storage conditions are not available (4) e g in certain geographic localities It may be noted also that acidification of formulas increases the likelihood that such milk will be used for infant feeding rather than for general family consumption In areas of the world where there is a general food shortage there is a danger that foods intended for infants may be consumed by other members of the family

Composition of Infant Formulas with Respect to Future Health Some Current Problems

The composition of infant formulas should also be looked upon with respect to future health of the baby An example of a current problem is the addition of sucrose to infant formulas This has not only bearing on the importance of keeping the mouth of the infant as free from sucrose as possible after the erup tion of the teeth in order to reduce the de velopment of caries Furthermore it is im portant not to accustom the children to a sweet taste in this way the craving for snacks and other sugar containing products later on in life could perhaps be prevented In addition it has been questioned whether an excessive intake of carbohydrates could not provoke an in creased insulin production which might be come permanent (13)

Another similar example concerns the rela tionship between infant diet and the develop ment of atherosclerosis a problem that during recent years has been the subject of growing interest (for references see 8 11)

Our knowledge so far about this relationship does not seem to argue for a marked change in the fat composition of infant formulas The main reason for this is the gener lly good experience of nutrition of infants (not breastfed) ccord ng to present principles Minor modifications of the f tty c d composition can however not be regarded as unjustifiable p ov ded they are based on valid experi mental studies and do not imply any harm to the baby Such a modification that has been discussed is a reduction to very low values of lauric and myristic acids as these fatty acids have in animal studies been shown to be strongly atherogenic (10 0) The same fatty acids have also been shown to be most potent in raising the serum cholesterol level in the human being (? 7) Another possi ble modification is a moderate increase of linoleic acid (e g. to 6% of total calories i e to the same value as is found in the breastmilk of mothers whose diet is rich in polyunsaturated fat) together these two changes will have a slightly depressing effect on the serum cholesterol level (6)

Although thus minor modifications could be discussed with respect to the relationship be tween infant (and childhood) diet and the de velopment of atherosclerosis later on in life certainly much more research remains to be done before we are ready for general state ments on this point

Enrichment of Infant Formulas

Generally speaking one could say that if there is a possibility to prevent deficiency disorders in infants through enrichment of infant formulas this possibility should be consid ered In practice however enrichment in volves often not only nutritional but also tech nological problems (e g influence on keeping quality) Enrichment of infant formulas is thus an example of a field where a research cooper ation between university and industry sci entists is desirable

Ethical Aspects on Sales Promotion

During recent years the ethics of sales promo tion have in many countries come under lively discussion especially with respect to possible conflicts vis a vis the propaganda for breast feeding It is true that most infant formula producers say in their advertising today that human milk is best for the baby It is how ever important that the producers in their sales promotion behave in such a way that this statement will have a real meaning and not appear to be hypocrisy It would be of great advantage if one could agree upon certain basic ethical rules These rules should protect

Table 1 *Main components and H^+ regulating processes in acid–base economy*

Components	Processes
1 Acid/base potentials of the diet	Gastrointestinal acid/base absorption
2 Intestinal processes	
3 Tissue growth	Endogenous H production
4 Intermediate metabolism	
5 Variation in compartmental size	Renal tubular HCO_3 reabsorption
6 Intercompartmental redistribution	Transcellular acid/base transport
7 Kidney function	Net acid excretion

In an individual with a normal acid–base balance (NAB) the net acid input to the kidney (NAI) influenced by components 1–6 is equal to the net acid excretion from the kidney (NAE) influenced by component 7 or

$$NAB = NAI - NAE$$

From Lindquist & Svenningsen 1973

An infant formula should of course not contain any ingredient that during a special period of life could be harmful to the baby e g gluten The introduction of gluten was discussed by ESPGA[1] at a round table conference already in 1969 in Interlaken and the conclusion was no gluten before the age of 4 months (12)

Furthermore infant formulas should not contain ingredients even if they are harmless if they have no meaning in infant nutrition Such ingredients could mislead the consumer about the quality and the nutritional value of the product This refers especially to starting formulas An example of an ingredient that according to my opinion should be discussed in this respect is honey so far no convincing evidence has been presented about the nutritional meaning of including honey in such formulas

In discussing ingredients in infant formulas consideration should also be given to acidification of milk

The disadvantages of acidified formulas have been discussed especially during recent years against the background of our present knowledge about the less well developed buffer capacity of the kidney during the first weeks of life It is now known that lactic acid (l form) as well as citric acid are normal metabolites of the body The capacity of newborns to metabolize these acids is limited when given in great amounts However acidified formulas do not contain such acids in great amount in relation to the homeostatic capability of the young infant Furthermore it is quite clear that acidification of the formula is a minor factor as compared to other factors of the total acid–base economy of the body provided dl forms are not used Table 1 shows a survey of different factors operating in this economy (9) From Fig 3 it is quite evident that the most important factor is surplus (excess) protein This figure shows the incidence of late metabolic acidosis in relation to protein intake in early life in preterm infants (appropriate for gestational age) this was found to be 10 3% 24 5% and 37 5% at a protein intake of 2 4 g 3 3 g and 5 7 g/kg/d respectively (16)

Another drawback of acidification of infant formulas has been shown to be somewhat adverse influence on fat absorption (5) which in turn could lead to an adverse effect also on mineral absorption especially calcium It should however be noted that other factors probably are of greater importance with respect to fat absorption in young babies In summary these are the fatty acid composition of the fat the amount of fat fed and the triglyceride structure of the fat (19) The fat absorption is also to a great extent depending on the age (5) and the maturity of the infant in prematures fed with cow s milk formula (impaired) fat absorption has been found to be correlated with low bile acid levels (14)

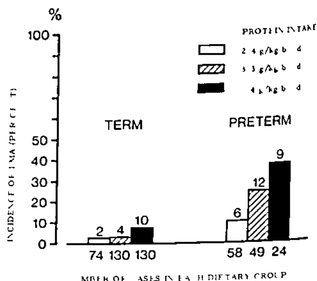


Fig 3 Incidence of Late Metabolic Acidosis (LMA) during the first weeks of life in term and preterm infants in relation to protein intake The figures below the columns give the total number in each group and figures above the numbers with LMA An infant was considered to have LMA whenever a base excess value below −8 0 mEq/l was found and confirmed twice (after 24 and 48 hours) From Lindquist & Svenningsen 1973

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[1] European Society for Pediatric Gastroenterology

metabolic acidosis in term preterm and small for gestational age infants in relation to dietary protein intake *Acta Paediatr Scand 62* 1 1973

17 — Postnatal development of renal hydrogen ion excretion capacity in relation to age and protein intake *Acta Paediatr Scand 63* 721 1974

18 United Nations System *Protein Advisory Group Statement No 23* Promotion of special foods (infant formula and processed protein foods) for vulnerable groups (July 18 1972 revised November 8 1973)

19 Widdowson E M The effect of the amount and kind of fat in the diet of young infants on their absorption of calcium magnesium and phosphorus *Collett Nutrition Symposia* September 1969 p 104 P M Bye og Snorre Oslo 1970

20 Wissler R W & Vesselinovitch D Recent advances in the understanding of the pathogenesis and the reversal of atherosclerosis *In Sommers Pathology Annual* vol 4 p 313 Appelton-Century Crofts New York 1969

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Key words Infant feeding infant formulas formula composition

the mothers against unduly persuasive advertising for formula products, especially starting formulas

The rules clearly need to be more strict in the third world (the preindustrialized countries) than in economically advanced countries In the former countries any kind of advertising that could give the mother the impression that an infant formula is as good for the baby as her breast milk must be avoided This applies above all to starting formulas Very often the mothers cannot afford to continue—when once started—to feed their babies formula products as instructed by the manufacturers They then dilute the formula products too much, which means that the babies are not given calories and essential nutrients in sufficient amounts leading to malnutrition Furthermore, bottle feeding under defective hygienic circumstances often leads to diarrhoea which in turn contributes to the development of malnutrition The Protein Advisory Group of the United Nations System also emphasizes the critical importance of breastfeeding under the sociocultural and economic conditions which prevail in many developing countries (18)

The following proposals are made

1 Preferably only adapted formulas should be recommended during the first month(s) of life Beside fullfilling certain nutritional requirement it is desirable that such formulas have an appearance and a consistency resembling human milk If they contain starch (or Schleim or gum preparations) they will have a thicker consistency which makes the mother believe that such a formula gives a better satiety feeling than breast milk When the babies cry—which they often do—the mothers believe that this depends on hunger She is then tempted to buy such a formula believing that this will be better for her baby

2 The term humanized should be abandoned and replaced with adapted

3 Advertising about starting formulas should preferably be directed to professional personnel doctors, nurses, dietitians, nutritionists, etc rather than to the consumers (or more correctly expressed to their mothers)

4 For reasons of hygiene and expense referred to above advertising must especially be restricted in developing countries

REFERENCES

1 American Medical Association Council on Foods and Nutrition Improvement of the nutritive quality of foods General policy *JAMA 225* 1116 1973

2 Arvidson G & Malmros H Studies on the influence of dietary fat on the fatty acid composition of serum lipids in humans *Z Ernaehrungswiss 11* 105 1972

3 Ernahrungskommission der Deutschen Gesellschaft für Kinderheilkunde Einteilung der Säuglingsmilchnahrungen auf Kuhmilcheiweissbasis Milchnahrungen für reifgeborene gesunde Säuglinge im ersten Lebenshalbjahr *Monatsschr Kinderheilkd 122* 761 1974

4 Fomon S J *Infant Nutrition* W B Saunders Company Philadelphia London and Toronto 1974 2nd ed

5 Fomon S J Ziegler E E Thomas L N Jensen R L & Filer L J Excretion of fat by normal full term infants fed various milks and formulas *Am J Clin Nutr 23* 1299 1970

6 Greenberg L D & Wheeler P Influence of fatty acid composition of infant formulas on the development of atherosclerosis and on the lipid composition of blood and tissues II Further lipid studies *Nutr Metab 14* 100 1972

7 Hegsted D M McGandy R B Myers M L & Stare F J Quantitative effects of dietary fat on serum cholesterol in man *Am J Clin Nutr 17* 81 1965

8 Lindquist B Die Ernahrung des Kindes im Hinblick auf die Verhütung der Atherosclerose Padiat Fortbild *Praxis 42* 42 1975

9 Lindquist B & Svenningsen N W Acid-base economy of the young infant *Cs Pediatr 28* 399 1973

10 Malmros H & Wigand G Experimental atherosclerosis *Bibl Nutr Dieta (Basel) 6* 139 1964

11 McBean L D & Speckmann E W An interpretive review Diet in early life and the prevention of atherosclerosis *Pediatr Res 8* 837 1974

12 Meeuwisse G W Diagnostic criteria in coeliac disease *Acta Paediatr Scand 59* 461 1970

13 Muller H R New quality criteria for infant formula feeding *In* Milk and Lactation *Mod Probl Paediatr 15* 213 1975

14 Signer E Murphy G M Edkins S & Andersson Ch M Role of bile salts in fat malabsorption of premature infants *Arch Dis Child 49* 174 1974

15 Svenningsen N W Lindquist B & Meeuwisse G W Nutrition and homeostasis with special reference to kidney function *Proceed XIII Intern Congr Pediatr* Wien *II* 509 1971

16 Svenningsen N W & Lindquist B Incidence of

metabolic acidosis in term preterm and small for gestational age infants in relation to dietary protein intake *Acta Paediatr Scand* 62 1 1973

17 — Postnatal development of renal hydrogen ion excretion capacity in relation to age and protein intake *Acta Paediatr Scand* 63 721 1974

18 United Nations System *Protein Advisory Group Statement No 23* Promotion of special foods (infant formula and processed protein foods) for vulnerable groups (July 18 1972 revised November 28 1973)

19 Widdowson E M The effect of the amount and kind of fat in the diet of young infants on their absorption of calcium magnesium and phosphorus *Collett Nutrition Symposia* September 1969 p 104 P M Bye og Snorre Oslo 1970

20 Wissler R W & Vesselinovitch D Recent advances in the understanding of the pathogenesis and the reversal of atherosclerosis *In Sommers Pathology Annual* vol 4 p 313 Appelton-Century Crofts New York 1969

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CONTROL OF RESPIRATION IN NEWBORN BABIES

III Developmental Changes of Respiratory Depth and Rate Responses to CO_2

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ABSTRACT Bodegård G (Department of Paediatrics Karolinska Institutet S t Goran s Children s Hospital Stockholm Sweden) Control of respiration in newborn babies III Developmental changes of respiratory depth and rate responses to CO_2 Acta Paediatr Scand 64 684 1975 —Thirteen healthy babies with PM ages varying between 32 and 43 weeks were investigated with regard to respiratory depth and rate changes on exposure to 4% CO_2 in air Two different types of responses were seen above 37 weeks of postmenstrual age the changes of depth and rate corresponded mostly to what is known in the adult human with a rate increase appearing only when the tidal volume had increased markedly (Type A responses) The preterm babies (i e under 37 weeks of PM age) responded mostly with a prompt rate increase without any preceding increase of the tidal volume (Type B response) The results indicate that the preterm baby in contrast to fullterm babies and adults may be dependent on the pulmonary vagal mechanoreceptor system for the regulation of the breathing in eupnoe

KEY WORDS Newborns respiration respiratory reflexes Hering Breuer mechanoreceptor regulation carbon dioxide

Respiratory reflexes connected to the pulmonary and the thoracic wall mechanoreceptor systems change in strength with maturation between 30 and 40 weeks of postmenstrual age (PM age) in the human baby Thus the Hering Breuer inflation reflex shows an increasing–decreasing development during this period and the thoracic wall mechanoreceptor system shows a principally increasing development of its strength simultaneously (2 3) The increase in strength of the thoracic wall system i e of the power generation of the respiratory muscles to airway occlusion may simply be interpreted as reflecting an increasing ability with age to maintain the ventilation against an increase in respiratory load The interpretation of the developmental change of the Hering Breuer inflation reflex on the other hand seems more difficult since the physiological significance of the pulmonary vagal mechanoreceptor system in general and the Hering Breuer inflation reflex in particular in the human is on the whole a matter of discussion (6 8–11 15 16)

The Hering Breuer inflation reflex has at least two different functional aspects since the effect of a lung inflation is dependent on where in the respiratory cycle it is done (6) Inflation at the height of the inspiration or during the expiratory phase results in a prolongation of the expiration (i e the classical Hering Breuer inflation reflex) (5 16) but if an inflation is done during the inspiratory phase the result is a termination of the inspiration (6) There is a linear relation between the prolongation of the expiration and the volume of the inflation

causing it (6) The inspiration on the other hand is dependent on where during the inspiratory phase the inflation is done so that the volume threshold decreases from the onset of the inspiration (6)

Studies by Clark & von Euler (6) in mature cats and adult humans indicate that the inspiration terminating effect of the Hering Breuer inflation reflex takes part in the regulation of the respiratory rate and depth changes provoked by CO_2 rebreathing In the adult human the increase of the ventilation on CO_2 is initially achieved by an increase only of the depth of the breathing cycles Not until the tidal volume has increased to 1 5–2 times the eupnoic values there is a rate increase seen The rate increase indicates that the inspiration terminating effect of the Hering Breuer inflation reflex is activated The afferent link in this reflex is thus assumed to be vagal and originating in the slowly adapting pulmonary stretch receptors of the classical Hering Breuer reflex (1 6) In the adult human there thus appears to be a functional threshold for the inspiration terminating effect of the Hering Breuer inflation reflex and when this is reached the further increase of the ventilation on CO_2 exposure is achieved by a combination of rate increase and a volume increase This means that this reflex probably is not of physiological importance in eupnoe in the adult human which is in line with what others have reported (11 15 16) The findings of Clark & von Euler motivated further elucidation of the Hering Breuer inflation reflex and its possible functional significance during the development in the human baby In the present study the functional threshold for ventilatory rate increase on exposures to increased amounts of CO_2 in the respired air was thus studied The investigations were performed on babies between 32 and 43 weeks of postmenstrual age (PM age) an age period during which the pulmonary vagal system i e the expiration prolongation effect of the Hering Breuer inflation reflex undergoes changes (2)

In our earlier studies we found that there is a time coincidence between the decreases in strength of the expiration prolongation effect of the Hering Breuer inflation reflex and the increase in strength of the reflex response to airway occlusion (3) This led to the suggestion that there may be an interaction between the two mechanoreceptor systems (3) It is known from animal studies that the diaphragmatic respiratory activity and thus the abdominal breathing excursions can be connected to the vagal respiratory regulation and that the respiratory activity of the intercostal muscles and thus the thoracic breathing excursions can be connected to the thoracic wall mechanoreceptor system (7 14) Since our earlier studies show that there are changes with maturation in both these parts of the respiratory regulatory systems the abdominal as well as the thoracic breathing excursions were followed separately in the present study

MATERIAL AND METHODS

Thirteen babies clinically healthy at the time of the investigations were studied The experiments were performed at varying postnatal ages but never earlier than 24 hours after birth The age of the babies at the time of the investigations is expressed as the PM age viz the sum of the gestational and the postnatal age The PM ages varied from 32 to 43 weeks There was a good correlation between the estimated PM age and the neurological behaviour and reflex pattern in all the babies studied Four of the babies were studied at more than one occasion (Table I) Babies with PM ages under 37 weeks will be referred to as preterm in the following and babies with PM age over 37 weeks as fullterm

The respiration was recorded as the intra pleural pressure changes and as the changes of the thoracic and abdominal circumferences A saline filled polyethylene catheter was introduced via a nostril to the lower third of the esophagus The intraesophageal pressure changes reflecting the intra pleural pressure changes (12) were recorded by a pressure transducer (4 327 L 221 + subunit 1 Devices Instruments Ltd England) The thoracic and abdominal circumference changes were recorded by two mercury strain gauges (Mercury in rubber strain gauges transducer 25 81 Devices Instruments Ltd England) applied around the thorax at the mammillary level and the abdomen immediately above the umbilical level The esophageal pressure recording was used preferably as an indicator for deciding the maximal point of inspiration which approximately coincides with the point of the lowest intra pleural pressure recorded during the breathing cycle This was necessary since the thoracic (and abdominal) breathing deflections in line with what others have

Table 1 *Data of infants examined Nine babies were examined once two were examined twice, one was examined three times and one baby five times*

Case		Birth weight (g)	Length at birth (cm)	Gestational age (weeks)	PM age at experiment (weeks)
I	Male	1 300	40	30	32
II	Male	1 290	42	30	33–34
					37
					39–40
III	Male	1 950	44	34	34
					35–36
					36
					37–38
					38
IV	Female	1 640	42	31–32	35–36
V	Female	2 170	47	34	34
					35–36
VI	Male	1 990	41	35	37
					38
VII	Male	2 430	46	36	38
VIII	Female	3 000	50	40	40
IX	Male	3 560	52	40	40
X	Female	4 150	50	38	41
XI	Male	5 050	55	41–42	41–42
XII	Female	3 410	49	42–43	42–43
XIII	Male	3 330	50	42	42–43

reported (13) could not always be identified as inspiratory or expiratory (see Results) The esophageal recording was then used as a reference

Since only the relative changes of the thoracic and abdominal breathing amplitudes were considered important for the aim of the present study the calibration of the Hg in rubber strain gauges was individually set in each baby to give recording deflections of convenient sizes No recordings of absolute volumes or volume changes were thus undertaken The thoracic and abdominal circumferential changes and the esophageal pressure changes were displayed on a four channel high speed pen recorder (Type M 4 Devices Instruments Ltd England)

The recordings were done when the babies had adapted to the registration equipment The CO_2 exposures were undertaken only when the babies were calm and lying in a supine position Thus when the babies from visual assessment were in eupnoe a rubber face mask was loosely attached to the face of the baby and perfused with 4% humidified CO_2 in air mixture of room temperature at a flow rate of 3 l per minute If the baby showed signs of increasing wakefulness such as movements or face grimaces when the mask was attached or during the exposure the experiment was discarded since then an arousal effect was likely to have added to the experimental situation The degree of wakefulness and its fluctuations were thus clinically assessed The CO_2 exposures were terminated when there was an increase of the respiratory rate noted in all experiments with a few exceptions (see further under Results) The aim to investigate the inspiration terminating effect of the Hering Breuer inflation reflex was considered fulfilled once the baby had responded with a rate increase and a standardization of the duration of the exposure time was thus not considered necessary The durations of the CO_2 exposures turned out to vary greatly

In all babies repeated CO_2 exposures were done and a total number of 166 acceptable recordings were achieved in the 13 babies The number of exposures in each single baby at each occasion of investigation varied from 2 to 1[illegible] (see Table 2) This variability had to be accepted since sedative preparations of the babies could not be used due to ethical reasons and in order not to artificially interfere with the nervous reactibility No blood gas studies were performed The babies were investigated at random in relation to feeding and ordinary nursery treatment The investigations were undertaken with the babies in their habitual situation i e incubator and bed respectively Caution was taken to prevent body temperature changes during the investigations

The mothers of the babies were asked for permission before the experiments were undertaken and the parents could be present at the investigations

RESULTS

There were two principally different ways of responding to the CO_2 exposures in the respiratory depth and rate changes In the following these will be referred to as Type A and Type B responses

Type A This response is characterized by a gradual and steady increase of the amplitude of the thoracic and abdominal breathing excursions A rate increase appears only when the tidal volume increase is pronounced as reflected in the recordings of the circumference changes of the chest and the abdomen The method of registration used did not allow any absolute quantitating of the tidal volume increase In some experiments the CO_2 exposure was interrupted before there was any rate increase seen but then only after a pronounced increase of the amplitude had been achieved Fig 1 shows a Type A response

Type B This response is characterized by a prompt increase of the rate without any preceding change of the amplitude Fig 2 shows Type B responses

In addition to these two types of responses there were also seen mixed responses Type M which showed fluctuations between the

Table 2 *The appearance of the different types of respiratory depth and rate responses to exposures to 4% CO_2 in air in 13 babies investigated at varying PM ages*

Nine babies have been investigated once and four repeatedly The material has been divided into five groups according to PM age The same baby can appear in one group several times because it has been examined repeatedly within that PM age period

Child no	PM age at investigation in weeks	No of expts	Type of response expressed in parts and percent: Type A	Type M	Type B
Period no 1 (32–34 weeks of PM age)					
I	32	12	3/12 (25%)	3/12 (25%)	6/12 (50%)
II	33–34	3	0/3 (0%)	1/3 (33%)	[illegible]/3 (67%)
III	34	16	0/16 (0%)	3/16 (19%)	13/16 (81%)
V	34	5	2/5 (40%)	3/5 (60%)	0/5
Mean percentage appearance of response			16%	34%	50%
Period no 2 (35–36 weeks of PM age)					
III	35–36	11	1/11 (10%)	5/11 (45%)	5/11 (45%)
III	36	6	4/6 (67%)	0/6 (0%)	2/6 (33%)
V	35–36	8	2/8 (25%)	3/8 (37 5%)	3/8 (37 5%)
IV	35–36	8	1/8 (12 5%)	1/8 (12 5%)	6/8 (75%)
Mean percentage appearance of response			28%	[illegible]4%	48%
Period no 3 (37–38 weeks of PM age)					
II	37	8	0/8 (0%)	3/8 (37 5%)	5/8 (62 5%)
III	37–38	5	2/5 (40%)	2/5 (40)	1/5 (20%)
III	38	14	8/14 (58%)	3/14 (21%)	3/14 (21%)
VI	37	4	0/4 (0%)	2/4 (50%)	2/4 (50%)
VI	38	10	8/10 (80%)	2/10 (20%)	0/10 (0%)
VII	38	9	7/9 (78%)	2/9 (22%)	0/9 (0%)
Mean percentage appearance of response			42%	32%	26%
Period no 4 (39–40 weeks of PM age)					
II	39–40	8	7/8 (88 5%)	1/8 (12 5%)	0/0 (0%)
VIII	40	6	3/6 (50%)	3/6 (50%)	0/0 (0%)
IX	40	12	7/12 (58%)	4/12 (33%)	1/12 (9%)
Mean percentage appearance of response			65%	32%	3%
Period no 5 (41–42 weeks of PM age)					
X	41	7	4/7 (57%)	3/7 (43%)	0/7 (0%)
XI	41–42	[illegible]	2/2 (100%)	0/2 (0%)	0/0 (0%)
XII	42–43	7	4/7 (57%)	1/7 (14%)	2/7 ([illegible]9%)
XIII	42–43	5	5/5 (100%)	0/5 (0%)	0/0 (0%)
Mean percentage appearance of response			79%	14%	7%

characteristics of the two clearcut types of responses

In addition to the findings regarding the respiratory depth and rate changes on the CO_2 exposures there were seen differences in the thoracic/abdominal breathing excursions between the two types of responses too

In the Type A responses the thoracic and abdominal respiratory excursions are always in phase and congruent viz the chest and abdomen expand in phase and with the point of maximal expansion coinciding with the point of the lowest intrapleural pressure reflecting the height of the inspiration This

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Mean percentage appearance of response			28%	24%	48%
Period no 3 (37–38 weeks of PM age)					
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III	37–38	5	2/5 (40%)	2/5 (40)	1/5 (20%)
III	38	14	8/14 (58%)	3/14 (21%)	3/14 (21%)
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VIII	40	6	3/6 (50%)	3/6 (50%)	0/0 (0%)
IX	40	12	7/12 (58%)	4/12 (33%)	1/12 (9%)
Mean percentage appearance of response			65%	32%	3%
Period no 5 (41–42 weeks of PM age)					
X	41	7	4/7 (57%)	3/7 (43%)	0/7 (0%)
XI	41–42	2	2/2 (100%)	0/2 (0%)	0/0 (0%)
XII	42–43	7	4/7 (57%)	1/7 (14%)	2/7 (29%)
XIII	42–43	5	5/5 (100%)	0/5 (0%)	0/0 (0%)
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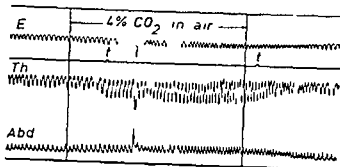


Fig 1 Tracings of the intraesophageal pressure swings (*E*) and the thoracic (*Th*) and abdominal (*Abd*) circumference changes during spontaneous non affected breathing and exposure to 4% CO_2 in air in a baby (No III—see Table 1) at 38 weeks of PM age Negative intraesophageal pressure changes deflections downwards Increase of thoracic circumference deflections downwards and increase of abdominal circumference deflections upwards (Paper speed 150 mm/min) Duration between each time marking (*t*) 60 secs

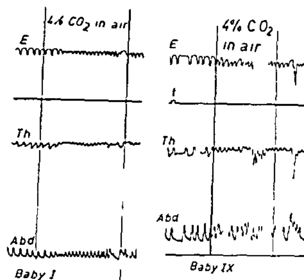


Fig 2 Tracings of the intraesophageal pressure swings (*E*) and the thoracic (*Th*) and abdominal (*Abd*) circumference changes during spontaneous non affected breathing and exposure to 4% CO_2 in air in two babies of 37 (No I—see Table 1) and 40 (No IX—see Table 1) weeks of PM age respectively Negative intraesophageal pressure changes deflections downwards Increase of thoracic circumference deflections downwards and increase of abdominal circumference deflections upwards (Paper speed 150 mm/min) Duration of time marking (*t*) 1 2 sec

type of thoracic abdominal breathing excursion pattern will in the following be called stable

In the Type B response the thoracic and abdominal breathing excursions are very different compared to the situation at hand in the Type A response The abdominal amplitude either remains unaffected or decreases simultaneously with the rate increase but there *is never any increase of the amplitude* The thoracic breating excursions are incongrous but in phase with the abdominal ones or they are totally inverted viz causing negative deflections in inspiration This type of thoracic abdominal breathing excursion pattern will be called *unstable* The compliance of the thoracic wall is obviously high in the unstable excursion pattern resulting in a more or less pronounced partial collapse of the chest with downward pulling of the ribs in inspiration

The Type A response was seen most often in the fullterm babies and the Type B response in the preterm babies The distribution of the different types of responses according to their relative occurrence at different PM ages is diagrammatically shown in Fig 3 For statistical reasons only the results from the first occasion of investigation in each baby are included in this diagram The occurrence of each type is expressed in percent of all the experiments done at the first occasion of investigation in each baby and is plotted against the PM age of the baby at the day of investigation Table 2 gives the figures corresponding to Fig 3

Fig 4 shows a diagram constructed similarly but including only the results from the experi

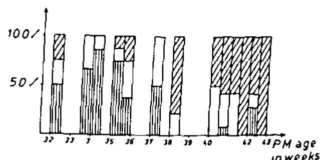


Fig 3 The appearance (in percent) of the three different types of responses regarding changes in depth and rate to exposure to 4% CO_2 in air in 13 different babies investigated at different PM ages Vertically lined fields Type B Unfilled fields Type M Diagonally lined fields Type A For description of types see text For data corresponding to this diagram see Table 2

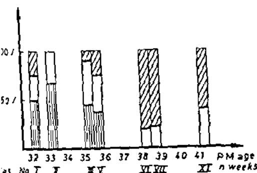


Fig 4 The appearance in percent of the three different types of responses regarding changes in depth and rate to exposure to 4% CO_2 in 7 of the babies which at these occasions of investigation were more than one but less than three weeks of *postnatal* age Vertically lined fields Type B Unfilled fields Type M Diagonally lined fields Type A For description of types see text For data see Table 2

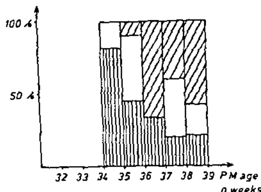


Fig 6 The appearance in percent of the different types of responses to exposure to 4% CO_2 in air in one baby (No III—see Table 1) investigated at five different PM ages Vertically lined fields Type B Unfilled fields Type M Diagonally lined fields Type A For description of types see text For data see Table 2

ments performed on the babies with postnatal age more than one but less than 3 weeks The figures corresponding to this diagram can be read from Table 2

Fig 5 shows a cross sectional diagram including all the 166 CO_2 exposures done The material of babies has been divided into 5 groups according to the PM age at the time of

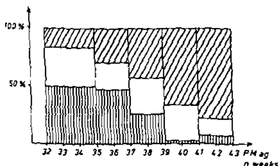


Fig 5 Cross sectional diagram showing the developmental change in appearance of the three different types of response regarding changes in respiratory depth and rate (for description of types see text) to exposure to 4% CO_2 in air in 13 babies investigated at varying PM ages The total number of 166 exposures has been divided into five groups according to the PM ages of the babies at the day of investigation For full description of the numbers of babies exposed and the distribution of the types of responses in the individual babies in each group see Table 2 Vertically lined fields Type B Unfilled fields Type M Diagonally lined fields Type A

the investigation Each type of response is expressed as its mean appearance in percent of the total number of exposures in each group Table 2 gives the number of experiments and the distribution of the types of responses in the individual babies in each of the 5 PM age groups

From the diagram in Figs 3 4 and 5 it can be seen that all three types of responses can occur at all maturity levels and in most of the individual babies However with increasing PM age there is a shift from a relative dominance of the Type B response to a relative dominance of the Type A response This developmental pattern is not essentially changed if the Type M responses are referred either to the Type A or Type B response respectively

Fig 6 shows the results from one baby studied at several occasions at different developmental stages The same general tendency to a shift from the Type B to the Type A dominance with increasing PM age is seen in this single baby as in the material as a whole

DISCUSSION

Babies between 32 and 43 weeks of PM age respond to exposures to 4% CO_2 in air with

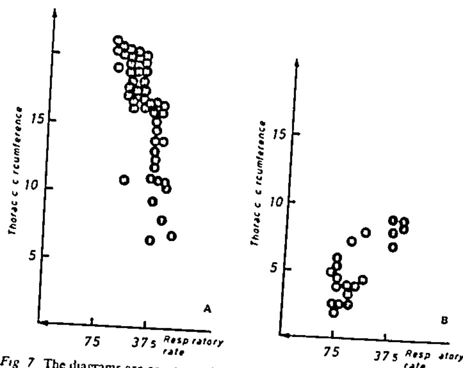


Fig 7 The diagrams are constructed on the single breath relation between the thoracic amplitude and the respiratory rate On the ordinate is the thoracic strain gauge measure of the thoracic circumference (expressed in mm on the recording) and on the abscissa is the respiratory rate The results underlying these two diagrams derive from two CO_2 exposures in one baby (No III—see Table I) investigated at 38 weeks of PM age The figures in circles correspond to the successive number of the single breath from the start of the CO_2 exposure A This response is characterized by the amplitude increase (Type A response) B This response is characterized by the rate increase (Type B response)

respiratory depth and rate changes of different types The type of response is mainly dependent on the PM age and thus the maturity of the baby

It is not possible to evaluate the quantitative changes of the pulmonary ventilation connected to the different types of responses achieved on CO_2 exposures since no spirometric studies were done From the general outlook of the recordings of the Type A responses it may be accepted that this type represents an increase of the ventilation The Type B response cannot similarly be interpreted as undoubtedly reflecting an increase of the ventilation This is however not principally important since the aim of this study was fulfilled once the baby had reacted with an increase of the rate It was thus principally unimportant what kind of quantitative blood gas changes that had provoked the rate increase or what kind of quantitative changes regarding the ventilation that had resulted

The Type B response anyway shows that—preferably—the preterm baby reacts with a prompt rate increase on exposure to a changed respiratory gas situation which provokes a demand for increased ventilation

If the assumption is correct that a rate increase achieved by exposure to CO_2 is due to termination of the inspirations via the Hering Breuer inflation reflex the findings of the present study shows that the pulmonary vagal mechanoreceptor regulation of the breathing undergoes marked changes between 32 and 43 weeks of PM age In the preterm babies the threshold for rate increase on CO_2 exposure is mostly low or absent (reflected in the Type B response) With increasing PM age there is a change to a relative dominance of a type of response similar to the one seen in the adult

Fig 7 shows the relation between the thoracic breathing amplitude and the respiratory rate in the single breaths during a typical Type A and Type B response in one

baby The Type A diagram shows the initial amplitude increase without rate increase and the following combined amplitude and rate increase This diagram corresponds to the V_T vs $1/T_I$ (tidal volume/inverted inspiratory duration) diagram reported by Clark & von Euler from CO_2 rebreathing experiments in adult humans (6) There is thus seen a Range I (only volume increase) and a Range II (combined volume and rate increase) in the Type A response In Range II the rate increase is assumed to be dependent on the terminations of the inspirations via the Hering Breuer inflation reflex and the decrease of the volume threshold for inspiration terminations from the onset of inspiration characteristic for the Hering Breuer inflation reflex is seen (6) In the Type B diagram there is no amplitude threshold for the rate increase This indicates that the rate regulation mechanism underlying this type of response can be of eupnoic importance

The amplitude/rate relation in the Type B response does not fit the characteristics for the Hering Breuer reflex described by Clark & von Euler This could theoretically be due to the central nervous system of the preterm baby interpreting inspiration interrupting impulses from the pulmonary stretch receptors differently On the other hand the pulmonary vagal impulses may in fact be different in the Type B response The differences of the thoracic/abdominal breathing excursions in the two types of responses indicate that the regulation of the thoracic wall stability is different in the two types of responses It then follows that the physical properties of the lungs and thus the stimulation of the pulmonary mechanoreceptors is likely to be different in the two types of responses The pulmonary vagal afferent input to the respiratory center is thus likely to be different in the two types of responses The Type B response could be due to hyperpnoe elicited from the lung irritant receptors (16) and the Hering Breuer *deflation* reflex would then be the reflex underlying the Type B response rather than the Hering Breuer inflation reflex Such an alternative explanation would still not alter the interpretation of the results of the present study that the preterm baby seems to be more dependent on the pulmonary vagal mechanoreceptor system for its depth and rate regulation than the fullterm baby and the adult

In an earlier study on the pulmonary vagal mechanoreceptor system it was shown that the expiration prolongation effect of the Hering Breuer inflation reflex is weak in the most immature babies and increases in strength to a maximum around 36–38 weeks of PM age and thereafter declines in strength again The interpretation of the present results indicate that the pulmonary vagal mechanoreceptor system appears to be of a strong functional importance in the most immature babies and to decrease in strength with increasing PM age The reason for this contradictory result may be that two independent aspects of the pulmonary vagal mechanoreceptor system are investigated in the former and the present study The present study does not however offer any possibility to evaluate the absolute strength of the reflex system it only indicates that the functional threshold for the rate increase on CO_2 exposure is low or absent in preterm babies and that increasing PM age changes the situation towards a regulation similar to what is known in the adult It then follows that if the pulmonary vagal mechanoreceptor regulation is poorly developed in the most immature babies reflected in the weak expiration prolongation effect of the Hering Breuer inflation reflex (2) but still is of a high functional importance reflected in the low threshold for rate increase this may offer one possible explanation as to why the respiratory regulation is so brittle in the very immature baby

A number of findings of interest in connection with the above presented discussion and interpretations were obtained during the registrations between the CO_2 exposures and will be presented in a future publication (4)

REFERENCES

1 Adrian E D Afferent impulses in the vagus and their effect on respiration J Physiol 79 332 1933

2 Bodegård G Schwieler G H Skoglund S & Zetterstrom R Control of respiration in newborn babies I The development of the Hering Breuer inflation reflex Acta Paediatr Scand 58 567 1969

3 Bodegård G & Schwieler G H Control of respiration in newborn babies II The development of the thoracic reflex response to an added respiratory load Acta Paediatr Scand 60 181 1971

4 Bodegård G Control of respiration in newborn babies IV Rib cage stability and respiratory regulation In manuscript

5 Breuer J Die Selbststeuerung der Atmung durch den Nervus Vagus Sber Akad Wiss Wien 58 909 1868

6 Clark F J & von Euler C On the regulation of depth and rate of breathing J Physiol 222 267 1972

7 Coombs H C & Pike F H The nervous control of respiration in kittens Am J Physiol 95 681 1930

8 Guz A Noble M I M Trenchard D Cochrane H L & Makey A R Studies on the vagus nerves in man their role in respiratory and circulatory control Clin Sci 27 293 1964

9 Guz A M Noble M I M Widdicombe J G Trenchard D & Mushin W W The effect of bilateral block of vagus and glossopharyngeal nerves on the ventilatory response to CO_2 of conscious man Resp Physiol 1 206 1966

10 Guz A M Noble M I M Eisle J H & Trenchard D Experimental results of vagal block in cardiopulmonary disease In R Porter (ed) Breathing Hering Breuer Centenary Symposium Churchill London 1970

11 — The role of vagal inflation reflexes in man and other animals In R Porter (ed) Breathing Hering Breuer Centenary Symposium Churchill London 1970

12 Mead J & Milic Emili J Theory and methodology in respiratory mechanics with glossary of symbols In W O Penn & H Rahn (eds) Handbook of Physiology Sec 3 Respiration Vol 1 Am Physiol Soc Washington D C 1964

13 Miller H C & Behrle F C Respiratory patterns in newborn infants as determined by airflow and pneumographic studies and their possible relationship to a deficient vagal mechanism Pediatrics 10 ?7? 1952

14 Shannon R & Zechman F W The reflex and mechanical response of the inspiratory muscles to an increased airflow resistance Resp Physiol 16 61 1972

15 Widdicombe J G Respiratory reflexes in man and other mammalian species Clin Sci 21 163 1961

16 — Reflexes from the lungs and the respiratory tract Acta Physiol Pol 3 Supplement ? 1971

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DIAGNOSTIC VALUE OF DISACCHARIDE TOLERANCE TESTS IN CHILDREN

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ABSTRACT Krasilnikoff P A Gudmand Høyer E and Moltke H H (From the Department of Paediatrics and the Medical Gastroenterological Department B University of Copenhagen Gentofte Hospital 2900 Hellerup Denmark) Diagnostic value of disaccharide tolerance tests in children Acta Paediatr Scand 64 693 1975 —The diagnostic value of oral lactose and sucrose tolerance tests was investigated in 61 children A total of 105 oral disaccharide tests were carried out When the rise in blood sugar was low the same disaccharide was, as a control measure instilled directly into the small intestine through a tube This was carried out in 40 cases *In 21 patients the rise in blood sugar following the two forms of* administration was correlated with the disaccharidase activity in a peroral small intestine biopsy The incidence of false-positive oral lactose tests was between 23 and 30% that of false-positive oral sucrose tests between 24 and 33% A border value of 20 mg per 100 ml in the rise in blood glucose within the first hour following a direct intra intestinal administration affords a very satisfactory distinction between patients with and without disaccharide malabsorption Blood glucose determinations exceeding one hour were found to be without diagnostic value

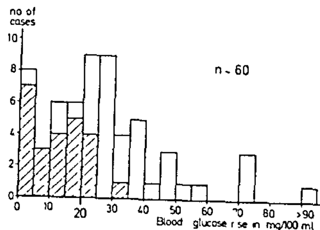
KEY WORDS Disaccharide tolerance test lactose sucrose intestinal biopsy

Tolerance tests with oral administration of the disaccharides lactose and sucrose followed by determination of the rise in blood glucose constitute the most common screening test for malabsorption of lactose and sucrose respectively In children a rise in blood glucose of less than 20 mg per 100 ml is usually considered an indication of malabsorption of the disaccharide in question (12)

In our department these tests are included in the routine examinations and the number of flat blood glucose curves thus registered has been surprisingly large However in a large number of patients the tolerance tests were not followed by attacks of diarrhea and abdominal symptoms and malabsorption of that particular disaccharide thus seems an improbable cause of the patient s dyspeptic symptoms It is possible however that these flat disaccharide tolerance curves constitute false-positive results due to a slow gastric emptying rate

During a period of almost 2 years we have therefore attempted a repetition of all such *oral disaccharide tolerance tests* viz tests showing a rise in blood glucose of less than 20–25 mg per 100 ml but in these cases by direct instillation of the disaccharide into the duodenum through a tube The results have also been correlated with the disaccharide activity in a peroral biopsy from the small intestine

We have also determined the time at which following the administration of the disaccharide in question the rise in blood glucose was maximal



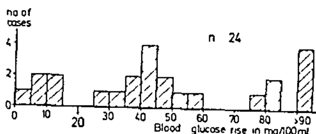


Fig 1 The results of oral lactose tolerance tests in 60 children In 24 cases with the lowest rise in blood glucose (hatched area) the test was repeated with intraduodenal instillation of lactose

MATERIAL

The material comprises 61 children ages ranging from 3 months to 14 years (mean 3 9 years median 2 8 years) 38 of whom were boys 23 girls They had all been admitted to hospital on account of chronic or intermittent diarrhea which had persisted for more than 3 months and/or due to a suspected malabsorption In 7 cases the patient was found to suffer from coeliac disease in 3 from specific sucrose malabsorption and in 2 from specific lactose malabsorption A total of 145 tolerance tests were carried out 40 of which by direct intraduodenal instillation The disaccharide activity in a peroral small intestine biopsy was determined in 21 cases

METHODS

Disaccharide tolerance tests Both oral and duodenal lactose and sucrose tolerance tests were carried out in the morning following an 8 hour fast The disaccharide in question was administered in a dose of 2 g per kilo body weight dissolved in a 10% solution In the case of direct intestinal instillation of the disaccharide solution the position of the tube was considered correct when an unhindered reflux of bile coloured small intestinal juice pH around 5 was obtained If any doubt as to the position of the tube arose a fluoroscopic control was carried out

Small intestine biopsy These biopsies were carried out in the vicinity of Treitz s ligament by means of Rubin pediatric multipurpose suction biopsy instrument The position of the instrument was controlled by fluoroscopy Two biopsies were removed In the one the activity of lactase sucrase and maltase was determined (?) The enzyme activities were expressed in International Units (I U) per g protein The protein content by Lowry method (8) The other biopsy was examined histologically by routine procedure

RESULTS

Lactose tolerance tests In 23 out of the 60 patients given an oral lactose tolerance test the rise in blood glucose was ≤20 mg per 100 ml and in 32 patients the rise was ≤25 mg per 100 ml In 24 of the patients showing the lowest rise in blood glucose the tests were repeated with intraduodenal instillation of lactose (Fig 1) This test showed a rise in blood glucose of ≤20 mg per 100 ml in only 5 patients Four of these patients suffered from coeliac disease and unspecific lactose malabsorption verified by biopsies and one patient suffered from specific lactose malabsorption also verified by biopsy

A correlation study between the maximal rise in blood glucose following oral ingestion as well as intraduodenal instillation and lactase activity in a peroral small intestine biopsy specimen was carried out in 18 cases (Fig 2)

A low lactase activity (<9 I U per g protein) as well as a low rise in blood glucose following both forms of administration was found in 4 out of 5 patients In one case the rise in blood glucose was higher than that expected from the lactase activity found This patient suffered from coeliac disease in which it is common knowledge that the changes in the small intestine and hence the reduction in disaccharidase activity are most pronounced in the proximal part of the small intestine and possibly confined to this localization In patients with a high lactase activity the rise in blood glucose was in 9 cases ≤20 mg per 100 ml when oral administration was used When using intraduodenal instillation the rise in blood glucose was normal in all cases except one in

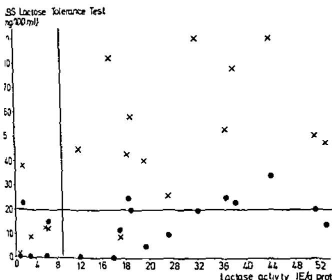
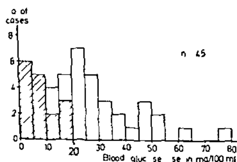


Fig. 2. Rise in blood glucose following oral (●) and intraduodenal (×) lactose tolerance tests correlated with the lactase activity in a peroral small intestine biopsy.

which the patient suffered from coeliac disease. In patients showing a rise in blood glucose above 20 mg per 100 ml following oral administration the rise in blood glucose was in all cases even higher following intraduodenal instillation of lactose.

Sucrose tolerance test. The results following sucrose tolerance tests were identical to those for lactose tolerance tests. Oral administration of sucrose was followed by a blood glucose rise ≤20 mg per 100 ml in 20 out of 45 patients (Fig. 3) and in 27 patients the rise in blood glucose was ≤25 mg per 100 ml. In 16 patients with a rise in blood glucose of ≤20 mg per 100 ml the test was repeated with intraduodenal instillation of sucrose. In all cases except 5 the rise in blood glucose was >20 mg per 100 ml, the exceptions comprising 3 patients with coeliac disease and unspecific sucrose malabsorption verified by biopsies and 2 patients with specific sucrose malabsorption also verified by biopsies.

Fig. 4 shows the correlation between the maximal rise in blood glucose following the two forms of administration and the sucrose activity in peroral small intestine biopsies in 13 patients. In cases with a low sucrase activity (<26 I.U. per g protein) the rise in blood glucose following the two forms of administration showed a good correlation. However one patient with coeliac disease showed a higher rise in blood glucose following intraduodenal instillation than was expected when considering the sucrase activity registered. In all of the



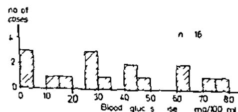
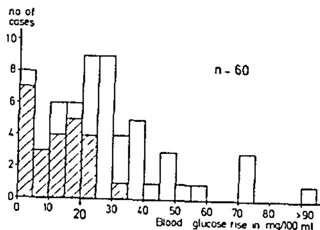


Fig. 3. The results of oral sucrose tolerance tests in 45 children. In 16 cases with the lowest rise in blood glucose (hatched area) the test was repeated with intraduodenal instillation of sucrose.



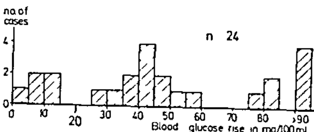


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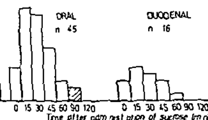
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g 6 Time at which the rise in blood glucose is maximal llowing oral and intraduodenal instillation of sucrose spectively

sucrose tolerance tests The tolerance tests were carried out in complete accordance with the procedures usually employed i e administration of 2 g disaccharide per kilo body weight dissolved in a 10% solution and frequent blood glucose determinations of capillary blood samples Determination of blood glucose was carried out by a glucose oxydase method

No publications are available concerning systematic investigations of the diagnostic value of either oral or intra intestinal disaccharide tolerance tests in children Various authors hold that in adults different factors may result in false–positive lactose tolerance tests

1 Use of venous blood instead of capillary blood for the determination of blood glucose (9 10)

2 Omitting blood glucose determination 15 or 20 minutes after the ingestion of lactose In this case the registration of an early peak value in blood glucose may be overlooked resulting in a false–positive lactose tolerance curve (1 10)

3 A slow gastric emptying rate (7 10)

The cause of the high incidence of flat oral disaccharide tolerance curves in the present study is probably a slow gastric emptying rate This conclusion is based on the finding of a normal rise in blood glucose following intraduodenal instillation in patients with a normal disaccharidase activity in the intestinal mucosa

Consequently one is doubtful as to the reliability of the results of available investigations as well as of the incidence of lactose malabsorption in children in whom the diagnosis has been established on the basis of oral lactose tolerance tests only However there is no doubt as to the fact that the incidence is high in children of races other than the Caucasian An investigation concerning Scandinavian children who are permanent residents of Ethiopia shows oral positive lactose tolerance tests in children to be a not uncommon occurrence (6) The investigations showed that 25% of 27 Scandinavian children presented with lactose malabsorption No small intestine biopsies were carried out In Scandinavia the incidence of lactose malabsorption is known to be definitely much lower than 25% In Denmark and in Sweden the incidence is between 1 and 6% in adults (4 2) and in Finland the incidence among schoolchildren is 6% (13) The abovementioned incidence of lactose malabsorption among Scandinavian children in Ethiopia is of the same order as the incidence of false–positive tests in the present study and may undoubtedly be thus explained

A high incidence of false–positive tests is further indicated by the fact that many children in whom lactose malabsorption had been diagnosed on the basis of an oral lactose tolerance test had no attacks of diarrhea and/or abdominal symptoms following the test (6 11 14) It seems unlikely that no symptoms should appear in children with lactose malabsorption after their ingestion of a dose of lactose as large as 2 g per kilo body weight In an adult weighing 75 kilos this would correspond to the ingestion of 150 g lactose or approx 3 litres cow s milk In adults with lactose malabsorption oral ingestion of 50 or 100 g lactose almost invariably results in diarrhea and abdominal symptoms (3 5 2)

The maximum rise in blood glucose occurred within the first hour after ingestion both in

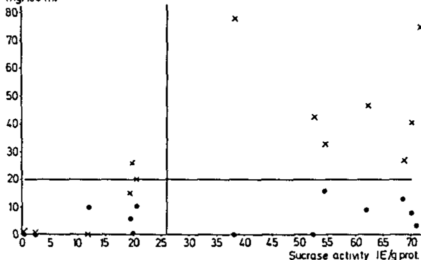


Fig 4 Rise in blood glucose during oral (●) and intraduodenal (×) sucrose tolerance test correlated with the sucrase activity in a peroral small intestine biopsy

7 patients showing a normal sucrase activity the rise in blood glucose was <20 mg per 100 ml following oral administration Intraduodenal administration showed normal figures which were in all cases above 25 mg per 100 ml

If the 4 patients showing a flat blood glucose curve following oral administration of lactose and in whom the lactose absorption had not been controlled by intraduodenal lactose instillation were actually suffering from lactose malabsorption 14 out of 60 patients examined (23%) would show a false flat oral lactose tolerance curve If the opposite had been the case 18 out of 60 patients (30%) would have shown a false flat oral lactose tolerance curve

Correspondingly control by direct intraduodenal instillation of sucrose was omitted in 4 patients with flat blood glucose curves following oral administration of sucrose If the flat curve following the oral sucrose tolerance test in these patients had been a true expression of their condition i e an actual sucrose malabsorption the result would have been a false flat curve in 11 out of 45 patients (24%) If the opposite had been the case the result in 15 out of 45 patients (33%) would have been a false flat curve following the oral sucrose tolerance test

The time following administration of lactose and sucrose respectively, at which the rise in blood glucose reaches its maximum is seen in Figs 5 and 6 This peak value was reached within the first hour after administration irrespective of the form of administration Three cases are exceptions to this rule one in the case of oral administration of lactose two in the case of oral administration of sucrose However in all 3 cases the maximal rise in blood glucose was <20 mg per 100 ml

DISCUSSION

The incidence of false-positive results following oral administration of disaccharides is in this material 23–30% in the case of lactose tolerance tests and 24–33% in the case of

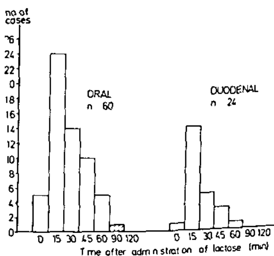


Fig 5 Time at which the rise in blood glucose is maximal following oral and intraduodenal instillation of lactose respectively

Acta Pædiatr Scand 64 699-702 1975

STUDIES ON THE EFFECT OF ORALLY ADMINISTERED AGAR ON THE SERUM BILIRUBIN LEVEL OF PREMATURE INFANTS AND MATURE NEWBORNS

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F WILCKEN and K HOEHNE

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ABSTRACT Windorfer A Jr Kunzer W Bolze H Ascher K Wilcken F and Hoehne K (University Children s Hospital Freiburg i Brg West Germany) Studies on the effect of orally administered agar on the serum bilirubin level of premature infants and mature newborns Acta Paediatr Scand 64 699 1975.—The effect of orally administered agar on the serum concentration of bilirubin was tested in 366 premature and newborn infants For this purpose two different concentrations of agar were added to the milk for 8–10 days On none of the groups tested did the serum level of bilirubin show a significant decrease after administration of agar Therefore the attempt to lower the level of serum bilirubin in premature and newborn infants by inhibiting enteric reabsorption by means of adsorbents must be considered a failure

KEY WORDS Premature infants serum bilirubin treatment with agar hyperbilirubinemia newborn exchange transfusion

1963 Brodersen et al (2) found a markedly creased content of indirect bilirubin in the ieces of newborns At the same time these ithors were able to demonstrate a considera ly increased activity of β glucuronidases in ie faeces of *newborns as compared to adults* ince bilirubin which is almost exclusively onjugated is excreted with the bile into the ntestine (6) the authors assumed that biliru m diglucuronide is uncoupled in the intestine f newborns According to investigations of *Lester & Schmid (6) unconjugated bilirubin* can be rapidly reabsorbed from the intestine and reach the circulation Brodersen et al (2) considered this fact as one of the possible causes of the development of hyperbili rubinemia in newborns Consequently they recommended *inhibition* of the reabsorption of uncoupled bilirubin by drugs to achieve reduction of the serum level of bilirubin Kunzer and co workers (3 4) and Ulstrom & Eisenklom (9) took up this sugges tion and made therapeutic attempts with acti *vated charcoal Later Poland & Odell (8) add* ed agar to the feedings of the first days of life An attempt was also made with oral adminis tration of cholestyramine (5) Whereas the therapeutic attempts with activated charcoal and cholestyramine showed only slight suc cess *Poland & Odell* reported a definite bilirubin reducing effect of agar given orally

Since the number of children examined by Poland & Odell (8) was very small we wished to obtain better information on the usefulness of treatment with agar in a larger group of patients

the case of lactose and sucrose and also in the case of both the two different forms of administration However in 3 patients the peak value was only reached in the second hour after ingestion In these 3 cases the maximal rise in blood glucose was less than 20 mg per 100 ml thus no further information would have been obtained by a prolonged determination of blood glucose

In the present material a 20 mg per 100 ml rise in blood glucose following intraduodenal disaccaride administration—but not following oral administration—has proved an excellent measure for separating the patient group with a normal disaccharidase activity from the patient group with a reduced activity

CONCLUSION

The conclusion of this study is that a flat disaccharide tolerance curve following an oral test must be verified by a tolerance test with direct intra intestinal instillation of the disaccharide that blood glucose determinations exceeding 1 hour after ingestion are without diagnostic significance and may be omitted and that the figure 20 mg per 100 ml in the rise in blood glucose following an intraduodenal disaccharide tolerance test in a very satisfactory manner distinguishes between patients with malabsorption of the disaccharide in question and patients with a normal absorption of the same

ACKNOWLEDGEMENT

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Mrs D Rasmussen has skillfully performed the enzyme assays

REFERENCES

1 Bayless T M & Rosenweig N S A racial difference in incidence of lactase deficiency A survey of milk intolerance and lactase deficiency in healthy adult males *JAMA 197* 968 1966
2 Dahlqvist A Assay of intestinal disacchandases *Anal Biochem 22* 99 1968
3 Gudmand Høyer E & Jarnum S The diagnosis of lactose malabsorption *Scand J Gastroenterol 3* 1 9 1969
4 Gudmand Høyer E Dahlqvist A & Jarnum S Specific small intestinal lactase deficiency in adults *Scand J Gastroenterol 4* 377 1969
5 Gudmand Høyer E *Specific lactose malabsorption in adults* Thesis FADL s Forlag Copenhagen Aarhus and Odense 1971
6 Habte D Sterky G & Hjalmarsson B Lactose malabsorption in Ethiopian children *Acta Paediatr Scand 62* 649 1973
7 Kern F Jr & Struthers J E Jr Intestinal lactase deficiency and lactose intolerance in adults *JAMA 195* 927 1966
8 Lowry O H Rosebrough N J Farr A L & Randall R J Protein measurement with Folin Phenol reagent *J Biol Chem 193* 265 1951
9 McGill D B & Newcomer A D Comparison of venous and capillary blood samples in lactose tolerance testing *Gastroenterology 53* 371 1967
10 Newcomer A D & McGill D B Lactose tolerance tests in adults with normal lactase activity *Gastroenterology 50* 340 1966
11 Olatunbosum D A & Adadevok B K Lactase intolerance in Nigerian children *Acta Paediatr Scand 61* 715 1972
12 Recommendation on the performance of lactose tolerance tests *In* B Borgstrom A Dahlqvist & L Hambræus (eds) *Intestinal enzyme deficiencies and their nutritional implications* Almqvist & Wiksell Stockholm 1973 p 149
13 Sahi T Isokoski M Jussila J & Launiala K Lactose malabsorption in Finnish children of school age *Acta Paediatr Scand 61* 11 1972
14 Varavithya W Valyasevi A & Charuchinda S Lactose malabsorption in Thai infants *J Pediatr 78* 710 1971

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Table 2 Infants with bilirubin levels higher than 12 and 15 mg/100 ml and infants requiring blood exchange transfusion with and without agar therapy

	No of newborns with serum bilirubin level=12 mg/100 ml	No of newborns with serum bilirubin level=15 mg/100 ml	No of newborns with blood exchange transfusion
Group I prematures with agar	13 (34%)	5 (13%)	1 (3%)
Group II prematures with agar	18 (37%)	7 (16%)	4 (8%)
Group III prematures control	29 (43%)	12 (16%)	4 (5%)
Group IV newborns with agar	11 (46%)	4 (17%)	3 (12%)
Group V newborns control	5 (42%)	2 (17%)	1 (8%)
Group VI newborns with agar	23 (23%)	8 (8%)	1 (1%)
Group VII newborns control	22 (23%)	5 (4%)	2 (3%)

of authors had only studied a very small number of patients Our results are less impressive even than those of Maurer and co workers (7) and of Blum & Etienne (1) who noted an effect of the addition of agar at least from the 4th day of life on But as mentioned before the case numbers are so small that it seems doubtful whether the results obtained by these authors really are of conclusive value

The difference in the average bilirubin values of the mature newborns of groups IV and V and of groups VI and VII is probably due to the fact that the infants of groups IV and V were ill newborns of the Pediatric Clinic while the infants of the groups VI and VII were healthy newborns from the Gynecological Clinic

The daily measured hematocrit values showed a significant decrease in all groups However no significant difference was found from the first to the last day of observation between the individual groups on the same day The weight losses also showed no significant differences between the individual groups The condition of the faeces was not changed by the two different additions of agar in particular no lesions of the intestinal wall were found which could have caused bleeding as detected by testing the faeces with benzidine

The reports suggesting a reduction of the serum bilirubin level by addition of agar to the feedings could not be confirmed Therefore treatment with oral adsorbents is not advisable

REFERENCES

1 Blum D & Etienne J Agar in control of hyperbilirubinemia *J Pediatr 83* 345 1973
2 Brodersen R & Sparre Hermann 1 Intestinal reabsorption of unconjugated bilirubin A possible contributing factor in neonatal jaundice *Lancet II* 124? 1963
3 Kunzer W Schenk W & Vahlenkamp H Bilirubinabsorption aus Duodenalsaft durch Kohle *Klin Wochenschr 41* 1108 1963
4 Kunzer W Vahlenkamp H Jarre W & Fuss W Zur Kohletherapie des Neugeborenen Ikterus *Ann Paediatr ?03* 247 1964
5 Lester R Hammaker L & Schmid R A new therapeutic approach to unconjugated hyperbilirubinemia *Lancet II* 1257 1962
6 Lester R & Schmid R The mechanism of intestinal absorption of bilirubin *J Clin Invest 41* 1379 196?
7 Maurer H M Shumway C N Draper D A & Hossaini A A Controlled trial comparing agar intermittent phototherapy and continuous phototherapy for reducing neonatal hyperbilirubinemia *J Pediatr 8?* 73 1973

Table 1 *Total bilirubin concentrations in newborn infants (mg/100 ml)*

Day of life	Group I premature with agar $n=38$	Group II prematures with agar $n=44$	Group III prematures control $n=62$	Group IV newborns with agar $n=24$	Group V newborns control $n=12$	Group VI newborns with agar $n=104$	Group VII newborns control $n=99$
1		3 79±1 25	3 6±1 0			3 3±1 2	3 1±1 4
2	5 9±2 4	6 3 ±2 0	6 6±1 5	6 8±3 0	5 4±3 7	5 2±2 0	5 3±1 9
3	7 5±2 8	8 3 ±2 9	9 2±2 0	8 1±3 1	8 5±4 3	6 8±3 0	6 9±3 0
4	8 6±3 7	9 7 ±3 3	10 3±2 8	9 2±4 1	8 9±5 1	7 4±3 9	7 6±4 1
5	9 0±3 6	9 7 ±3 9	10 0±3 5	9 3±4 5	9 1±4 9	6 9±4 4	7 0±4 4
6	9 3±4 0	8 9 ±4 1	9 9±3 4	8 9±5 0	7 3±4 7	5 9±4 3	6 1±4 4
7	8 5±3 5	8 3 ±3 6	9 2±3 9	7 9±4 2	7 2±4 3	5 2±4 0	5 1±3 8
8	7 6±3 3	7 7 ±3 6	8 2±3 6	6 9±3 9	4 7±3 6		
9	6 8±3 0	6 9 ±3 2	7 6±3 3	6 3±3 5	5 1±3 8		
10	6 0±2 9	6 1 ±3 1	7 5±3 2	5 5±3 1	5 2±3 4		

MATERIAL AND METHODS

In a period of 14 months a total of 366 newborns and premature infants were examined The children were divided into the following groups

Group I Premature infants 1 400–2 500 g $n=38$ ½% agar added to the daily milk diet from the first milk meal onwards

Group II Premature infants 1 300–2 400 g $n=44$ 1 g agar added to the daily milk diet in 4 portions already from the first meal onwards

Group III Premature infants 1 400–2 500 g $n=62$ Control group without addition to diet

Group IV Mature newborns (Univ Children s Hospital) $n=24$ ½% agar added to the daily milk diet from the first milk meal onwards

Group V Mature newborns (Univ Children s Hospital) $n=12$ Control group without addition to the diet

Group VI Mature newborns (Univ Gynecological Clinic) $n=104$ 1 g agar added to the daily milk meal in 4 portions from the first meal onwards

Group VII Mature newborns (Univ Gynecological Clinic) $n=99$ Control group without addition to diet

The treatment was carried out according to the following scheme All children born from the first to the 15th day of each month were given agar in the concentration shown above children born from the 16th to the 31st day of each month served as controls We used Agar Merck p A Because of excessive thickening the agar was not boiled with the milk but added in powder form to the boiled milk while hot Capillary blood was obtained daily between 5 p m and 7 p m to determine the total serum bilirubin and the hematocrit The serum bilirubin was measured with a filterphotometer (A–0 bilirubinometer American Optical Corp) a hematocrit capillary tubeful was sufficient to determine the two parameters Furthermore weight and condition of the faeces were recorded daily the latter mainly for possible blood content as detected by benzidine

RESULTS AND DISCUSSION

Table 1 shows the mean values of the total bilirubin concentrations in the serum of all premature and mature newborns examined with the standard deviations The bilirubin values on the individual days do not significantly differ between groups within one weight class this means that with neither concentration of agar added to the food could a significant reduction of bilirubin be achieved in any of the groups Significant differences in the bilirubin values were only found as was to be expected between the premature and the newborn infants from the third day of life onwards but independently of the addition of agar Neither did a further breakdown of the values obtained lead to any changes in the negative result

In Table 2 the numbers of children of all groups with bilirubin values higher than 12 mg/100 ml and 15 mg/100 ml are shown as well as those requiring blood exchange transfusions Here again the number of children with high bilirubin values in the groups having received agar was not different from that in the control groups

Whereas Poland & Odell (8) noted a levelling off in the bilirubin values in a small group of patients who were given additional agar after 30 hours these results could not be confirmed by Maurer and co workers (7) nor by Blum & Etienne (1) However all three groups

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THE EFFECT OF CALCIUM INFUSIONS ON RENAL HANDLING OF AMINO ACIDS IN HYPOPHOSPHATEMIC VITAMIN D RESISTANT RICKETS

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ABSTRACT Colombo J P and Donath A (Department of Clinical Chemistry and Department of Paediatrics Inselspital University of Berne and the Division of Nuclear Medicine Department of Internal Medicine University of Geneva Switzerland) The effect of calcium infusions on renal handling of amino acids in hypophosphatemic vitamin D resistant rickets Acta Paediatr Scand 64 703 1975 —Amino acid clearances were studied in four patients with hypophosphatemic vitamin D resistant rickets The clearances of the single amino acids in the patients did not differ from that of control individuals for all amino acids tested The reabsorption of amino acids and phosphate was further investigated with the use of calcium infusions. Under these conditions there was a significant decrease in the filtered fraction of phosphate and amino acid excreted in the vitamin D resistant group suggesting a depression of parathyroid hormone secretion It seems likely as demonstrated in the vitamin D resistant group *that in this disorder the renal tubule is particularly sensitive to changes in parathyroid* hormone secretion in respect to amino acid reabsorption

KEY WORDS Amino acids vitamin D resistant rickets parathyroid hormone

Aminoaciduria is frequently observed in vitamin D deficiency rickets Amino acid clearances have been reported to be elevated depending on the age of the patients (4) and the stage of the disease (4 12) The renal hyperaminoaciduria found in vitamin D deficiency rickets is attributed by some investigators to a direct effect of vitamin D deficiency (5 16 17) and by others to a secondary hyperparathyroidism (12 14 30) In this context we examined the question whether renal handling of amino acids in hypophosphatemic vitamin D resistant rickets follows a mechanism similar to that in vitamin D deficiency rickets Since the infusion of calcium in vitamin D resistant rickets leads to an increased tubular phosphate reabsorption which might be mediated by influencing parathyroid hormone secretion (15 29 35) it was investigated if those observations also apply to amino acids This would suggest similar regulatory mechanisms of amino acid reabsorption in this disorder Amino acid reabsorptions were therefore measured before and after calcium infusions Since the influence of calcium infusions on renal phosphate reabsorption has already been studied phosphate was only determined to evaluate the effect of the calcium infusion (10)

Supported by the Swiss National Foundation for Scientific

MATERIAL

Patients

B E boy 7 3/4 years height 103 cm (~3 percentile) weight 0 5 kg (<10 percentile) The boy is of short stature and shows various signs of rickets such as marked

8 Poland R L & Odell G B Physiologic jaundice The enterohepatic circulation of bilirubin *N Engl J Med* 284 1 1971
9 Ulstrom R A & Eisenklom E The enterohepatic shunting of bilirubin in the newborn infant I Use of oral activated charcoal to reduce normal serum bilirubin values *J Pediatr* 27 27 1964

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ble 2 Data on renal function in vitamin D resistant rickets and control subjects before and er calcium infusion

R glomerular filtration rate Cl=clearance FF =filtered fraction excreted P=inorganic phosphate Pl=plasma amino acid

	Vitamin D resistant			Control subjects		
	Before Ca	After Ca	p	Before Ca	After Ca	p
FR (ml/min/1 73 m)	156±15	127±33	n s	105±22	96±7 0	n s
(mg/100 ml)	1 9±0 8	3 [illegible]±0 8	<0 05	4 6±0 6[b]	4 8±0 2	n s
(ml/min/1 73 m²)	[illegible]±9 9	10 1±9 1	<0 05	5 5±[illegible] 8	3 4± [illegible]	n s
F_P (%)	15 6±7 4	5 3±2 9[f]	<0 0[illegible]	7 5±5 0[a]	3 6±2 2	n s
L_{AA} (ml/min/1 73 m²)	3 3±2 9	0 9±0 8	<0 01	3 6±3 1	2 1±2 0	n s
F_{AA} (%)	3 6±1 9	0 6±0 8	<0 02	1 9±[illegible] 3	1 8±2 6	n s

Significance versus vitamin D resistant $p<0\ 002$
Significance versus vitamin D resistant $p<0\ 005$
Significance versus vitamin D resistant $p<0\ 005$
[d] Significance versus vitamin D resistant $p<0\ 005$
Boys (average) 14 2 girls (average) 20 0
[f] Boys 4 7 girls 7 0

control subjects This was also the case when the absolute values without correction for standard body surface area were considered The clearance of the total amino acids in the patients did not differ significantly from the values in the control subjects (Table 2) This was also observed for the clearance of the single amino acids (Table 3) When compared with the values established by Brodehl & Gellissen using the same analytical techniques (3) the mean values found for most of the amino acids in our patients show a definite upward trend though it could not be proved statistically

The calcium infusion influenced various parameters In the vitamin D resistant group a nificant decrease was noted in the total ino acid clearance compared with the con l group (Table 2) The degree of fall of the iction of filtered amino acids excreted within patients group after calcium infusion was istically significant for all amino acids (Ta 4) and amounted on average to 84% hereas this was not observed in the control oup In the normal control individuals the ajority of amino acids was not affected by e calcium infusion A significant diminution f the fraction of filtered phosphate excreted nd of the phosphate clearance occurred in all he vitamin D resistant cases after calcium in usion (Table 2) The plasma phosphate concentration increased in the patients whereas no changes were observed in the control individuals No statistical differences were found for the glomerular filtration rate within both groups after calcium infusion

DISCUSSION

An increased excretion of one or several amino acids in vitamin D resistant rickets has occasionally been reported in non familial and

Table 3 Amino acid clearances (ml/min/1 73 m²) in patients with vitamin D resistant rickets and control subjects

Only those amino acids are quoted which were excreted in all patients

Amino acids	Vitamin D resistant	Controls	Normal values
Threonine	2 8±1 6[illegible]	1 12±0 76	1 01±0 [illegible]4
Serine	1 83±0 92	1 7[illegible]±1 38	2 43±0 51
Glycine	10 33±4 07	6 08±[illegible] 98	4 4±1 41
Alanine	1 [illegible]0±0 44	0 98±0 64	0 82±0 38
Valine	0 35±0 13	0 [illegible]1±0 14	0 [illegible]3±0 11
Isoleucine	0 63±0 09	0 45±0 09	0 32±0 09
Leucine	0 95±0 37	0 31±0 21	0 49±0 15
Tyrosine	2 88±1 35	[illegible] 18±1 83	2 04±0 80
Phenylalanine	1 87±0 59	1 33±1 17	1 50±0 [illegible]9
Ornithine	0 75±0 17	0 91±0 73	0 38±0 1[illegible]
Lysine	1 80±0 86	0 86±0 65	1 16±0 38
Histidine	11 73±3 92	14 00±5 07	9 51±2 63
Arginine	0 45±0 10	0 17±0 13	0 [illegible]8±0 10

According to (3)

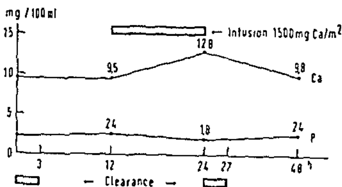


Fig 1 Scheme of the experimental procedure for the calcium infusion studies Plasma concentrations of cal cium and phosphate are indicated

costochondral junctions (rachitic rosary) carious teeth coxa vara and bowlegs He has large metaphyses with typical rachitic X ray findings There was no re sponse to normal doses of vitamin D The urine specific gravity was 1031 Since the parents of the child live in Italy the familiality of the disorder could not be ascertained

J M girl 6 1/4 years height 102 cm (<3 percentile) weight 19 6 kg (<25 percentile) The girl exhibits various rachitic symptoms including a caput quadratum rachitic rosary lordosis of the lumbar region marked inhibition of extension in elbows and knees coxa vara and genua valga She shows typical rachitic findings in the X rays The familiality of the disorder could not be assessed since the child lives with foster parents

K Ch boy 12 years height 130 cm (<3 percentile) weight 30 6 kg (<3 percentile) The boy is of short stature He has large metaphyses with typical rachitic X ray find ings The legs are slightly bowed The renal concentration capacity was normal (1003 mOsm/l)

K S boy 15 1/2 years brother of *K Ch* height 156 cm (<3 percentile) weight 49 7 kg (<25 percentile) He is also of short stature exhibiting signs of a rachitic ros ary coxa vara and large metaphyses His urinary con centration capacity was normal A familial incidence of the disease is present

A treatment with pharmacological doses (up to 250 000 U daily) of vitamin D did not improve the clinical and biochemical condition in these children

METHODS

Amino acid and phosphate clearance studies were performed in the 4 patients and in 4 control individuals 2 boys aged 12 years and 2 medical students We are well aware that adult persons may not represent adequate con trols in this study But in view of the circumstantiality of the experimental procedure we refrained from unsing fur ther children as controls The children were taken off of any vitamin D treatment 3 months before the clearance studies The calcium infusion was made according to the procedure described by Royer et al (28) The experiment started at 8 00 (o hour in Fig 1) in the morning with a 3 hour clearance period from 20 00 to 08 00 Ca gluconate at a dose of 1 500 mg/m² in saline was infused followed by a 3 hour clearance period The glomerular filtration rate (GFR) was measured using the single injection clearance technique with ^{51}Cr EDTA (7 33) which showed a close correlation with standard inulin clearance techniques (33 34) The urine collection period for the amino acid and phosphate clearance was 3 hours Blood samples were drawn in the middle of each clearance period Amino acids were determined using a Technicon amino acid analyzer with the technique of Efron (8) Serum was deproteinized with solid sulfosalicylic acid For urine an amount of the supernatant corresponding to a 0 5 min urine output was placed on the column Phosphorus was determined with the molybdenum blue method (27) and alkaline phosphatase by using *p* nitrophenol as substrate (27) Calcium was measured with an Eppendorf flame photo meter

Statistical methods

All data are expressed as means and standard deviations Differences were calculated with the Student s *t* test (27) *p* values below 0 05 were considered significant

RESULTS

All patients suffered from rachitic deformities particularly of the lower extremities with typ ical X ray findings in the epiphysial–metaphys ial regions In two of them a familial incidence of the disease could be ascertained Serum calcium was normal in all individuals while serum phosphorus was low The alkaline phosphatase activity was always above the normal value for the age group (Table 1) The phosphate clearance and the fraction of filtered phosphate excreted were elevated in the patients (Table 2) The glomerular filtra tion rate was significantly higher than in the

Table 1 *Values of inorg phosphate calcium and alkaline phosphatase in the plasma of pa tients with vitamin D resistant rickets during the experimental period*

	Age (y)	Plasma (mg/100 ml)		Alk phos phatase (IU/1 000 ml)
		P	Ca	
B E (m)	7¾	2 4–3 0	8 8–9 5	160–173
J M (f)	6¼	1 8–2 4	9 5–9 8	219–257
K S (m)	12	1 1–1 9	9 1–9 2	160–200
K Ch (m)	15¼	1 5–2 3	9 0–9 5	136–142
Normal range		3 5–5 5	9–11	36–140 13–45 (>15 y)

able 2 *Data on renal function in vitamin D resistant rickets and control subjects before and ter calcium infusion*

R= glomerular filtration rate Cl=clearance FF =filtered fraction excreted P=inorganic phosphate Pl=plasma amino acid

	Vitamin D resistant			Control subjects		
	Before Ca	After Ca	*p*	Before Ca	After Ca	*p*
R (ml/min/1 73 m²)	156±15	127±33	n s	105±22	96±7 0	n s
(mg/100 ml)	1 9±0 8	3 2±0 8	<0 05	4 6±0 6ᵃ	4 8±0 2	n s
(ml/min/1 73 m²)	24 2±9 9	10 1±9 1	<0 05	5 5±? 8	3 4±2 1	n s
(%)	15 6±7 4	5 3±2 9ᶠ	<0 025	7.5±5 0ᵈ	3 6±2 2	n s
A (ml/min/1 73 m)	3 3±? 9	0 9±0 8	<0 01	3 6±3 1	1±? 0	n s
AA (%)	3 6±1 9	0 6±0 8	<0 0?	1 9±2 3	1 8±2 6	n s

Significance versus vitamin D resistant $p<0\ 002$
Significance versus vitamin D resistant $p<0\ 005$
Significance versus vitamin D resistant p 0 005
ᵈ Significance versus vitamin D resistant $p<0\ 005$
Boys (average) 14 2 girls (average) ?0 0
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DISCUSSION

An increased excretion of one or several amino acids in vitamin D resistant rickets has occasionally been reported in non familial and

Table 3 *Amino acid clearances (ml/min/1 73 m²) in patients with vitamin D resistant rickets and control subjects*

Only those *amino acids* are quoted which were excreted in all patients

Amino acids	Vitamin D resistant	Controls	Normal values
Threonine	2 ?8±1 62	1 1?±0 76	1 01±0 24
Serine	1 83±0 9	1 7?±1 38	2 43±0 51
Glycine	10 33±4 07	6 08±2 98	4 24±1 41
Alanine	1 20±0 44	0 78±0 64	0 82±0 38
Valine	0 35+0 13	0 21±0 14	0 3±0 11
Isoleucine	0 63±0 09	0 45±0 09	0 3?±0 09
Leucine	0 95±0 37	0 31±0 21	0 49±0 15
Tyrosine	2 88±1 35	18±1 83	2 04±0 80
Phenylalanine	1 83±0 59	1 33±1 17	1 50±0 29
Ornithine	0 75±0 17	0 91±0 73	0 38±0 12
Lysine	1 80±0 86	0 86±0 65	1 16±0 38
Histidine	11 73±3 9?	14 00±5 07	9 51±? 63
Arginine	0 45±0 10	0 17±0 13	0 ?8±0 10

According to (3)

Table 4. *Fraction of filtered amino acids excreted in patients with vitamin D resistant rickets an control individuals before and after calcium infusions*

	Vitamin D resistant			Controls		
	Before	After	*p*	Before	After	*p*
Taurine	13.31±3.8	1.89±1.3	<0.01	6.51±3.8	7.23±2.13	n.s.
Threonine	1.40±0.95	0.34±0.1	<0.05	1.20±1.3	0.83±0.93	n.s.
Serine	2.11±1.35	0.42±0.28	<0.01	1.13±0.52	0.75±0.51	n.s.
Glutamic acid	1.30±1.23	0.24±0.16	<0.05	0.79±0.47	0.53±0.72	n.s.
Glycine	6.72±3.49	1.17±0.50	<0.01	4.34±2.40	3.70±1.71	0.05
Alanine	1.24±0.55	0.20±0.10	<0.01	0.71±0.22	0.54±0.36	0.05
Valine	0.27±0.24	0.08±0.05	<0.025	0.24±0.24	0.27±0.22	n.s.
Isoleucine	0.62±0.16	0.37±0.31	<0.05	0.31±0.21	0.51±0.78	n.s.
Leucine	0.45±0.72	0.19±0.18	<0.05	0.57±0.25	0.16±0.26	0.05
Tyrosine	3.16±2.38	0.54±0.42	<0.05	1.87±0.99	1.90±1.70	n.s.
Phenylalanine	1.79±1.78	0.33±0.25	<0.05	1.17±1.78	0.97±0.85	n.s.
Ornithine	1.33±0.61	0.16±0.14	<0.01	0.45±0.03	0.48±0.78	n.s.
Lysine	2.32±2.19	0.32±0.17	<0.01	1.13±0.49	0.63±0.46	n.s.
Histidine	17.06±10.6	2.98±2.35	<0.01	7.39±2.16	8.57±7.37	n.s.
Arginine	0.42±0.43	0.13±0.09	<0.01	0.26±0.04	0.14±0.09	0.05

familial instances of this disorder (9, 17, 21, 23, 32). The sensitivity of the analytical procedure employed plays an important role in the detection of amino aciduria. The observations of Lapatsanis et al. showed that paper chromatography may not be sensitive enough (21). With the use of column chromatographic techniques they detected definite amino aciduria in 3 of 4 patients observed (21). Using this technique Jonxis & Huisman in earlier studies measured the increased urinary output of threonine, serine, glycine, alanine, histidine and lysine (19, 20). In a later publication clearances of amino acids were reported to be normal, but no values were given (18). From our studies it would appear that in vitamin D resistant rickets amino acid clearances are elevated. Due to the elevated glomerular filtration rate in the presence of normal plasma amino acid concentrations, the glomerular load of amino acids was larger in the patients. In relation to the elevated load, however, and despite the higher fraction of filtered amino acids excreted, their reabsorption capacity was still sufficiently high to avoid hyperaminoaciduria. The reason for the elevated glomerular filtration rate in vitamin D resistant rickets is not clear. This phenomenon has also been discussed with respect to vitamin D deficiency rickets (4). It may be related to a deficiency in or unresponsiveness to vitamin D or to parathyroid hormone action (24).

Calcium infusions in men have also been used recently for the investigation of disorders of parathyroid function (15, 29, 35). Induction of hypercalcemia, particularly of the non ionized calcium (11), is known to suppress parathyroid hormone secretion (31). In addi tion to this main effect, however, some metabolic alterations may be attributed to a direct action of calcium itself. In dogs the infu sion into the renal artery caused a moderat reduction in the glomerular filtration rate an increased the tubular reabsorption c phosphate (22). A systemic venous infusion a carried out in the patients may however not b quite comparable in its direct effect on rena function to an application into the renal artery. Furthermore, calcium infusion influence calcitonin secretion. This might provoke a similar but temporary phosphaturic effect lik that produced by parathyroid hormone (1, 25). Despite these side effects, we think that a major effect of calcium infusions on rena handling of amino acids and phosphate is mediated through parathyroid hormone. Infu sion studies may therefore give indirect in formation on the still uncertain pathogenesi of phosphaturia, hypophosphatemia and re

nal amino acid handling in vitamin D resistant rickets If an increased parathyroid hormone activity is to be expected in vitamin D resistant rickets it should be influenced by calcium infusions

The significant improvement in phosphate clearance and in the fraction of filtered phosphate excreted after calcium infusion in the patients would indicate a depression of a parathyroid hormone secretion probably somewhat augmented A certain moderate degree of hyperparathyroidism in this disorder is rather probable although our findings cannot directly prove it Unfortunately reliable determinations of plasma parathyroid hormone levels were not available during the course of this study to document the concentration of circulatory immunoreactive hormone at which these changes occurred Reitz & Weinstein (26) as well as Levy et al were able to demonstrate increased levels of parathyroid hormone activity in individuals with untreated vitamin D resistant rickets which under calcium infusion decreased into the normal range (23) The degree of hyperparathyroidism however may not always be sufficient to explain the severity of phosphaturia (23) or as we think to provoke amino aciduria regularly in these cases

Some studies demonstrate that parathyroid hormone not only modulates the tubular reabsorption capacity for phosphate but probably also for amino acids Using micropuncture techniques of the kidney it could be demonstrated that parathyroid hormone injections in the rat were followed by a decreased amino acid reabsorption (13) Parathyroid hormone injections in vitamin D deficient children led to a reduced reabsorption of aminoacids (2 12) Hyperaminoaciduria has also been observed in primary hyperparathyroidism It is often reversible after removal of the overactive parthyroid tissue (6) Calcium infusions in our experiments not only enhanced the reabsorption of phosphate but also the reabsorption of all amino acids in the vitamin D resistant group All the transport systems for amino acids were involved (36) To our knowledge this has not yet been shown in vitamin D resistant rickets Since no changes were observed in normal individuals a direct effect of calcium on tubular reabsorption of amino acids seems rather improbable although it cannot be ruled out completely in the patients Our findings therefore rather suggest that the underlying cause may be an augmented acitivity of parathyroid horomone It may be unmasked by calcium infusions leading to an increased reabsorption of phosphate and amino acids only in the patients with vitamin D resistant rickets but not in normal individuals This leads us to speculate that the renal tubule as was demonstrated in the patients with vitamin D resistant rickets might respond in a particularly sensitive way to changes in parathyroid hormone secretion in respect to amino acid reabsorption Further investigations in normal individuals as well as in the disorders of vitamin D metabolism especially on the site of hormonal action in the kidney tubule are needed

REFERENCES

1 Ardaillou R Vaugnat P Milhaud G & Richet G Effets de la thyrocalcitonine sur l excretion renale des phosphates du alcium et des ions H chez l homme *Nephron 4* 298 1967

2 Brodehl J The effect of calcium and parathormone on renal handling of phosphate and free amino acids in normal hypoparathyroid and rachitic children *Europ Soc Pediat Res Ann Meeting* Stockholm 19 0

3 Brodehl J & Gellissen K Endogenous renal transport of free amino acids in infancy and childhood *Pediatrics 42* 395 1968

4 Brodehl J Kaas W P & Weber H P Vitamin D deficiency rickets Renal handling of phosphate and free amino acid *Pediat Res 5* 591 1971

5 Chisholm J J & Harrison H E Aminoaciduria in vitamin D deficiency states in premature infants and older infants with rickets *J Pediat 60* 206 196_

6 Cusworth D C Dent C E & Scriver C R Hyperparathyroidism and hyperaminoaciduria *Clin Chin Acta 41* 355 197

7 Donath A The simultaneous determination in children of glomerular filtration rate and effective renal plasma flow by the single injection clearance technique *Acta Pied atr Sc nd 60* 5 8 1971

8 Efron M L Quantitative estimation of amino acids in phys lc gical flu ds using a Technicon amino acid analyzer Autc mation in analytical chem stry Tech n on Sympos um New York 1965 p 637

Table 4 *Fraction of filtered amino acids excreted in patients with vitamin D resistant rickets and control individuals before and after calcium infusions*

	Vitamin D resistant			Controls		
	Before	After	*p*	Before	After	*p*
Taurine	13 31±3 8	1 69±1 3	<0 01	6 51±3 8	7 23±2 13	n s
Threonine	1 40±0 95	0 34±0 1	<0 05	1 20±1 3	0 83±0 93	n s
Serine	2 11±1 35	0 42±0 28	<0 01	1 13±0 52	0 75±0 51	n s
Glutamic acid	1 30±1 23	0 24±0 16	<0 05	0 79±0 47	0 53±0 72	n s
Glycine	6 72±3 49	1 17±0 50	<0 01	4 34±2 40	3 70±1 71	0 05
Alanine	1 ?4±0 55	0 20±0 10	<0 01	0 71±0 22	0 54±0 36	0 05
Valine	0 27±0 24	0 08±0 05	<0 025	0 24±0 24	0 27±0 22	n s
Isoleucine	0 62±0 16	0 37±0 31	<0 05	0 31±0 21	0 51±0 8	n s
Leucine	0 45±0 72	0 19±0 18	<0 05	0 57±0 25	0 16±0 ?6	0 05
Tyrosine	3 16±2 38	0 54±0 42	<0 05	1 87±0 99	1 90±1 70	n s
Phenylalanine	1 79±1 78	0 33±0 25	<0 05	1 17±1 78	0 92±0 85	n s
Ornithine	1 33±0 61	0 16±0 14	<0 01	0 45±0 03	0 48±0 8	n s
Lysine	2 32±2 19	0 32±0 17	<0 01	1 13±0 49	0 63±0 46	n s
Histidine	17 06±10 6	2 95±2 35	<0 01	7 39±2 16	8 57±7 37	n s
Arginine	0 42±0 43	0 13±0 09	<0 01	0 ?6±0 04	0 14±0 09	0 05

familial instances of this disorder (9 17 21 23 32) The sensitivity of the analytical procedure employed plays an important role in the detection of amino aciduria The observations of Lapatsanis et al showed that paper chromatography may not be sensitive enough (21) With the use of column chromatographic techniques they detected definite amino aciduria in 3 of 4 patients observed (21) Using this technique Jonxis & Huisman in earlier studies measured the increased urinary output of threonine serine glycine alanine histidine and lysine (19 20) In a later publication clearances of amino acids were reported to be normal but no values were given (18) From our studies it would appear that in vitamin D resistant rickets amino acid clearances are elevated Due to the elevated glomerular filtration rate in the presence of normal plasma amino acid concentrations the glomerular load of amino acids was larger in the patients In relation to the elevated load however and despite the higher fraction of filtered amino acids excreted their reabsorption capacity was still sufficiently high to avoid hyperaminoaciduria The reason for the elevated glomerular filtration rate in vitamin D resistant rickets is not clear This phenomenon has also been discussed with respect to vitamin D deficiency rickets (4) It may be related to a deficiency in or unresponsiveness to vitamin D or to parathyroid hormone action (24)

Calcium infusions in men have also been used recently for the investigation of disorders of parathyroid function (15 29 35) Induction of hypercalcemia particularly of the non ionized calcium (11) is known to suppress parathyroid hormone secretion (31) In addition to this main effect however some metabolic alterations may be attributed to a direct action of calcium itself In dogs the infusion into the renal artery caused a moderate reduction in the glomerular filtration rate and increased the tubular reabsorption of phosphate (22) A systemic venous infusion as carried out in the patients may however not be quite comparable in its direct effect on renal function to an application into the renal artery Furthermore calcium infusion influences calcitonin secretion This might provoke a similar but temporary phosphaturic effect like that produced by parathyroid hormone (1 25) Despite these side effects we think that a major effect of calcium infusions on renal handling of amino acids and phosphate is mediated through parathyroid hormone Infusion studies may therefore give indirect information on the still uncertain pathogenesis of phosphaturia hypophosphatemia and re

QUANTITATIVE DETERMINATION OF IMMUNOGLOBULINS LYSOZYME AND CERTAIN ELECTROLYTES IN BREAST MILK DURING THE ENTIRE PERIOD OF LACTATION DURING A 24 HOUR PERIOD AND IN MILK FROM THE INDIVIDUAL MAMMARY GLAND

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ABSTRACT Peitersen B Bohn L and Andersen H (The Children s Hospital Fuglebakken and the Department of Clinical Chemistry Bispebjerg Hospital Copenhagen Denmark) Quantitative determination of immunoglobulins lysozyme and certain electrolytes in breast milk *during the entire period of lactation for a 24-hour period and in milk from the individual mammary gland* Acta Paediatr Scand 64 709 1975.—During a period commencing at birth and lasting for up to 27 months 193 milk samples have been collected from 29 mothers The IgA globulin content was high immediately after birth averaging 2 7 arb U decreasing to 0 3 arb U within the first 2 to 3 weeks after birth then remaining almost constant for the rest of the lactational period In the case of IgG globulin similar results were obtained but the quantity was much smaller IgM globulin was demonstrated in small quantities during the first 3 weeks of lactation The lysozyme content varied considerably during the whole lactational period Individual variations were found for all the immunoglobulins while the concentration in the individual woman varied only slightly from day to day following in other respects the pattern described above In 19 mothers IgA IgG IgM, lysozyme and electrolyte content were determined in serum and in milk from the right and the left breast on the same day No difference in content was found between milk from the left and the right mammary gland A positive correlation was found between the concentrations of IgA and sodium chloride in milk between those of IgG in milk and serum and between those of lysozyme in milk and serum No variations were registered during the individual breast feeding nor for the 24 hour period as a whole

KEY WORDS Immunoglobulins lysozyme electrolytes human milk daily variations mammary gland

In recent years the proteins of breast milk especially immunoglobulins have been reviewed with increasing interest Only a few publications on quantitative determinations have appeared while the number of investigations concerned with qualitative determinations is much larger (3 7 8 9 10 11 22) During the period 1959–61 Hanson and co workers found 14 immunologically different precipitates in colostrum *i e the milk col*lected during the first 96 hours after birth 13 of these precipitates being present also in milk collected later in the lactational period Other investigations (18 23 31) have corroborated this and at present at least 30 different antigens have been demonstrated in colostrum 15 of which were also found in milk collected at later stages in the lactational period 18 out of the 30 antigens were similar to those found in serum (12 14 22) whereas

9 Fanconi G & Girardet P Familiarer persistieren der Phosphatdiabetes mit Vitamin D resistenter Rachitis *Helv Paediatr Acta 7* 14 1952
10 Field M H & Reiss E Vitamin D resistant rickets The effect of calcium infusion on phosphate reabsorption *J Clin Invest 39* 1807 1960
11 Fischer J A Binswanger U & Blum J W The acute parathyroid hormone response to changes in ionized calcium during phosphate infusions in the cow *Europ J Clin Invest 3* 151 1973
12 Fraser D Kooh S W & Scriver C R Hyperparathyroidism as the cause of hyperaminoaciduria and phosphaturia in human vitamin D deficiency *Pediatr Res 1* 425 1967
13 Gelke D Der Einfluss von Parathormon auf die Nierenfunktion I Tierexperimentelle Untersuchungen *Pfluegers Arch 323* 96 1971
14 Grose J H & Scriver C R Parathyroid dependent phosphaturia and aminoaciduria in the vitamin deficient rat *Amer J Physiol 214* 370 1968
15 Haas H G Metabolic criteria for primary hyperparathyroidism *Helv Med Acta* ? 91 1966
16 Hassanein E A & Patel H The parathyroid hormone and aminoaciduria in vitamin D deficiency rickets *Acta Paediatr Scand 56* 445 1967
17 Jonxis J H P Aminoaciduria and rickets *Helv Paediatr Acta 10* 245 1955
18 — Some investigations on rickets *J Pediatr 59* 607 1969
19 Jonxis J H P & Huisman T H J Amino aciduria in rachitic children *Lancet ii* 428 1953
20 — The renal element in rachitic amino aciduria *Lancet II* 513 1954
21 Lapatsanis P Athanasiadou Danelatou K & Karpouzas J Therapeutic studies in vitamin D refractory rickets *Isr J Med Sci 7* 537 1971
22 Lavender A R & Pullman Th Changes in inorganic phosphate excretion induced by renal arterial infusion of calcium *Amer J Physiol 205* 1025 1963
23 Lewy J E Cabana E C Repetto H A Canterbury J M & Reiss E Serum parathyroid hormone in hypophosphatemic vitamin D resistant rickets *J Pediatr 81* 294 197?
24 Pullman T N Lavender A R Aho J & Rasmussen H Direct renal action of purified parathyroid extract *Endocrinology 67* 570 1960
25 Rasmussen G Anast C & Arnaud C Thyrocalcitonin EGTA and urinary electrolyte excretion *J Clin Invest 46* 746 1967
26 Reitz R E & Weinstein R L Parathyroid hormone secretion in familial vitamin D resistant rickets *New Engl J Med 289* 941 1973
27 Richterich R *Klinische Chemie Theorie und Praxis* S Karger Basel Munchen Paris London New York and Sydney 1971 3 erweiterte Auflage
28 Royer P Habib R & Mathieu H *Problemes actuels de nephrologie infantile* Ed med Flammarion Paris 1963 p 19
29 Schott H Jobiz W Frailey J & Tyler F H Calcium infusion and phosphate deprivation tests in patients with primary hyperparathyroidism and with normocalcemia and nephrolithiasis *Metabolism 21* 205 1972
30 Scriver C R Kooh S W & Fraser D Aminoaciduria in vitamin D deficiency rickets and in disturbances of parathyroid function *J Pediatr 65* 1085 1964
31 Sherwood L M Potts J T Jr Care A D Hayer G P & Aurbach G D Evaluation by radioimmunoassay of factors controlling the secretion of parathyroid hormone *Nature 209* 5? 1966
32 Tobler R Prader A & Taillard W Die familiare Vitamin D resistente Rachitis (Phosphatdiabetes) *Helv Paediatr Acta 11* 204 1956
33 Truniger B Donath A & Kappeler M Simplified clearance techniques The single injection method and its modifications *Helv Med Acta 34* 116 1968
34 Voegeli B Riedwyl H Donath A & Oetliker O Comparison of glomerular filtration rate and effective renal plasma flow determinations obtained by a single injection technique and by means of a standardized clearance technique in children *Acta Paediatr Scand 60* 528 1971
35 Wibell L Johansson H & Werner I The use of a calcium infusion test in the diagnosis of hyperparathyroidism *Scand J Lab Clin Med 30* 183 197?
36 Young J A & Freedman B S Renal tubular transport of amino acids *Clin Chem 17* 245 1971

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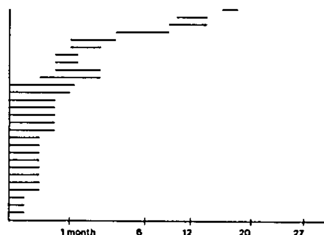


Fig 1 The distribution of the material during the period of lactation and the number of days during which the individual mother supplied milk Each line represents one mother

at least 12 were specific for milk (13 14 22) Furthermore pre colostrum i e milk secreted 3 to 4 weeks prior to death contained fewer antigen substances but in late pre colostrum and in colostrum the antigen content was almost identical both containing IgM in determinable quantities while only traces were found in milk collected after the third week of lactation On the other hand IgG and especially IgA are found in colostrum as well as in milk from the later stages of lactation In all quantitative determinations IgA is the predominant immunoglobulin in breast milk constituting 90% of all immunoglobulins in early colostrum and in milk from the first month of lactation IgG was found in considerably smaller amounts and IgM was found in determinable quantities only during the first week of lactation (2 25 27) Finally Underwood et al 1970 (31) examined the protein content in breast milk from Pakistani women the milk being collected within a period from the 6th week to 24 months after birth A distinct fall in protein content from the 6th week to the 6th month after birth was registered Following this the protein concentration remained stable for the following 12 months increasing slightly during the period 18 to 24 months after birth This late rise was not statistically significant

Several investigations concerning the content of electrolytes proteins amino acids and minerals in breast milk have been published (4 5 6 19 21) but only Chodirker et al (4) have carried out a simultaneous investigation of the serum content of these substances in the women in question IgA IgG and IgM as well as lysozyme and electrolytes have been determined in milk collected simultaneously

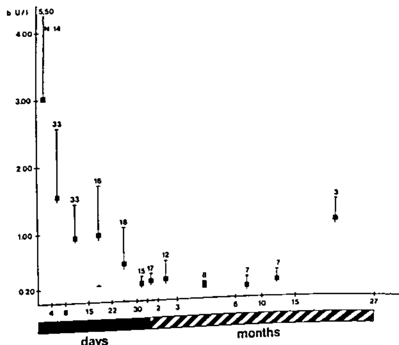


Fig 2 IgA content during the period of lactation which has been divided into groups The number of samples within each group is indicated by N The concentration of IgA is given in arbitrary Units based on the mass concentration values (g/l) given in the instructions from Behring Werke

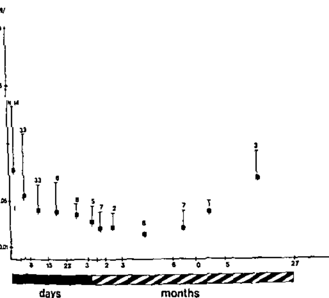


Fig 5 IgG content during period of lactation which has been divided into groups The number of samples within each group is indicated by *N*

reaching an almost constant value of 0 3 arb U/l after 2 to 3 weeks Towards the end of the lactational period the content increases slightly These findings hold true for the individual mother as well as for the material as a whole It is remarkable that the individual variations in IgA concentration are so great while the concentration in the individual case varies very little within the above mentioned pattern

The changes in the IgA concentration in breast milk simply during the lactational period are not merely a question of dilution as is evident from Table 1 and Figs 3 and 4 Here the total 24 hour secretion of IgA has been calculated in 10 mothers and the total secretion is seen to be high in colostrum while a decrease soon sets in i e in the course of 1–2 weeks Following this the content remains more or less constant for the remainder of the lactational period It should be noted that the

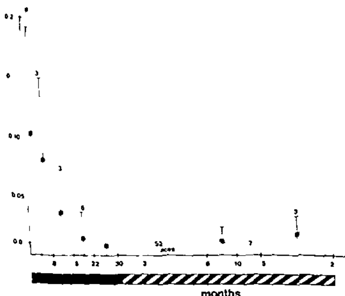


Fig 6 IgM content during period of lactation which has been divided into groups The number of samples within each group is indicated by *N*

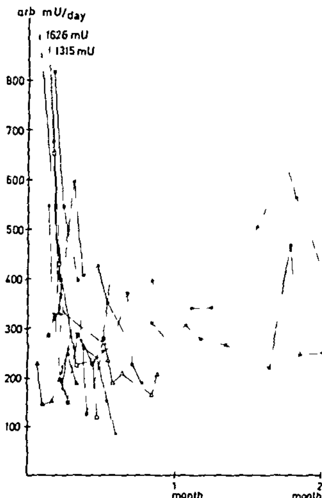


Fig 3 Total secretion of IgA in milk per day in 9 different mothers Samples are taken from first 2 months of lactational period IgA concentration is given in arbitrary Units (see test) ■—■=mother no 1 ▲—▲=no 2 △—△=no 3 ○—○=no 4 □—□=no 5 ●—●=no 6 ×—×=no 7 ×—=no 8 ■—■=no 9

munoglobulins and lysozyme For the electrolytes the precision expressed as coefficient of variation was 1% Commencing with the first 4 days after birth i e colostrum and continuing up to 27 months after birth 193 milk samples representing the entire period have been collected from 29 mothers Each mother supplied a varying number of milk samples viz from 1 to 16 collected at different times during the entire period of lactation However none of the mothers supplied milk for periods longer than 3 months in all as seen in Fig 1

The mothers were specially selected some because the child was hospitalized immediately after birth the remainder being mothers supplying milk to the Centre for Breast Milk at the Children s Hospital Fuglebakken In the latter group lactation was maintained longer than usual The majority of the samples were collected early in the lactational period while 4 mothers were able to supply milk for periods exceeding one year after birth One of the women collected milk samples in the beginning half way and at the end of a breast feeding repeating the procedure morning noon and night on the same day and repeating it again on 5 different days within the first and second month of lactation

Finally samples of milk were collected from each breast separately in 19 women Serum samples as well as the milk samples from the individual patient were collected at the same time on the same day while the time in relation to the period of lactation varied from woman to woman Determinations of sodium ion potassium ion chloride ion IgA IgG IgM and lysozyme were carried out for all samples

RESULTS

Fig 2 shows that the content of IgA in breast milk is high shortly after birth ranging from 0 48 arb U/l to 8 78 arb U/l mean 2 7 arb U/l The concentration then drops sharply

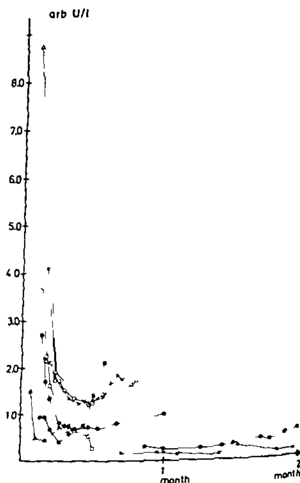


Fig 4 IgA concentration in milk from 9 different mothers Samples are all taken from first 2 months of lactational period IgA concentration is given in arbitrary Units (see test) ■—■=mother no 1 ▲—▲=no 2 △—△=no 3 ○—○=no 4 □—□no 5 ●—●=no 6 ×—=no 7 ×—=no 8 ●—●=no 9

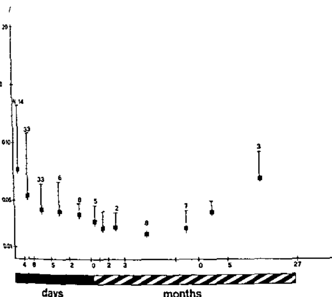


Fig 5 IgG content during period of lactation which has been divided into groups The number of samples within each group is indicated by *N*

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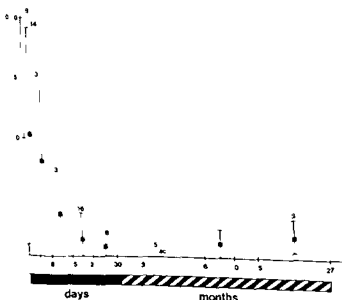


Fig 6 IgM content during period of lactation which has been divided into groups The number of samples within each group is indicated by N

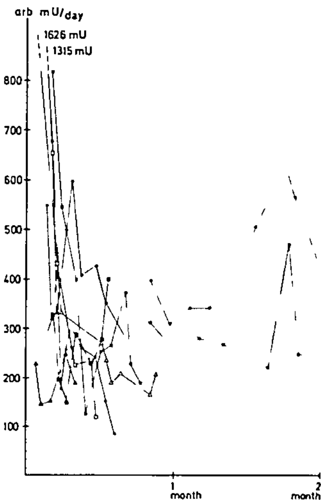


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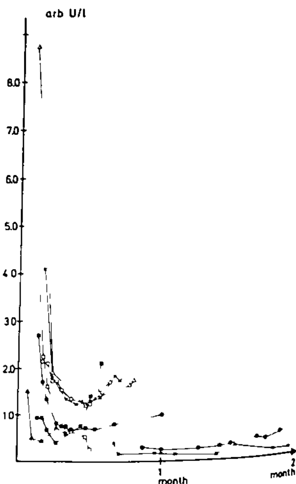


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Table 2 *Content in milk from right and left mammary gland and in serum on the same day (19 different persons)*

r=right l=left, s=serum

Pat no	Potassium ion (mmol/l)			Sodium ion (mmol/l)			Chloride ion (mmol/l)			IgA (arb U/l)			IgG (g/l)			Lysozyme (mg/l)		
	r	l	s	r	l	s	r	l	s	r	l	s	r	l	s	r	l	s
8	17 6	18 6	4 1	13 0	10 2	136	17	17	109	0 663	0 667	1 72	0 024	0 023	10 08	24 8	45 0	2 14
9	12 4	15 3	4 6	7 4	15 3	139	10	16	102	0 467	0 377	2 77	0 018	0 075	13 38	74 3	35 0	2 19
11	12 8	16 3	4 3	12 5	11 3	137	17	17	103	0 458	0 499	1 63	0 048	0 012	10 39	190 0	217 0	2 06
12	14 7	16 5	4 0	12 8	10 0	138	16	14	100	0 278	0 317	3 80	0 046	0 042	13 51	18 1	13 3	1 80
15	15 1	14 4	3 5	6 3	6 2	172	10	10	102	0 7 8	0 208	2 52	0 021	0 022	10 07	41 0	38 8	1 54
0	16 7	16 7	4 4	7 7	2 5	138	7	8	93	0 703	0 708	2 72	0 029	0 029	10 41	32 0	32 8	1 82
22	14 3	15 7	4 5	6 0	5 2	136	12	13	97	0 232	0 276	2 30	0 032	0 077	12 34	160 0	170 0	1 90
25	15 7	16 8	3 4	12 9	10.5	138	19	17	105	0 297	0 489	0 80	0 004	0 005	6 25	9 0	7 9	1 82
28	15 1	15 0	4 0	7 7	8 1	139	10	10	102	0 363	0 352	1 29	0 008	0 015	9 93	13 6	16 5	1 79
30	15 7	15 2	4 0	8 2	11 6	140	12	14	100	0 264	0 787	2 96	0 058	0 071	14 29	216 0	202 0	2 68
18	14 3	15 6	4 7	34.0	23 0	140	31	24	86	0 593	0.555	2 78	0 045	0 079	10 70	40 4	37 2	2 70
48	14 0	13 8	5 1	16 0	72 0	144	0	27	107	0 363	0 433	1 63	0 090	0 102	13 61	35 6	36 2	2 10
32	16 7	17 9	4 4	9 0	10 0	143	16	17	104	0 546	0 669	2 50	0 025	0 038	9 43	23 5	27 2	1 70
47	14 7	16 7	4 9	5 0	7 0	144	10	14	107	0 199	0 384	2 00	0 016	0 032	10 68	15 8	34 2	1 10
79	15 9	15 3	4 5	13 0	15 0	139	16	17	106	0 987	0 932	3 48	0 051	0 051	11 62	52 6	63 1	0 93
39	14 4	15 2	4 6	11 0	11 0	141	16	16	103	0 124	0 180	0 85	0 017	0 024	7 77	223 9	245 8	2 28
45	19 3	19 3	4 3	9 0	10 0	142	16	16	106	0 310	0 378	1 15	0 026	0 025	9 40	30 0	28 7	1 71
46	11 0	11 7	4 2	2 0	3 0	143	5	7	104	0 108	0 158	1 00	0 018	0 016	11 84	29 1	37 2	1 19
35	15 9	18 5	4 4	5 0	5 0	140	10	13	102	0 279	0 365	3 50	0 054	0 046	15 83	17 2	13 4	1 10

potassium and lysozyme were much higher in the milk samples than in the corresponding serum samples while the concentration of the other substances was considerably lower in milk than in serum in the individual person

The 24 hour variations of IgA IgG and IgM as well as lysozyme in the milk collected from one of the women appear in Table 3 No significant change in the content of the different immunoglobulins and lysozyme was found either in the course of a single breast feeding or for the 24 hours as a whole

DISCUSSION

The content of the immunoglobulins IgA IgG and IgM is in short high in colostrum decreasing rapidly during the first weeks of the lactational period Three to 4 weeks after birth only weak traces of IgM persisted while IgG and especially IgA were found in determinable quantities during the whole period of lactation even when this period lasted for more than 2

The primary cause of the individual variations and of the varying level of the concentration curves is probably an active secretion of certain immunoglobulins by the mammary gland (24 27 28 29 30 32) IgA globulin is found in a high concentration in various secretions for instance bile saliva bronchial and nasal secretion and spinal fluid indicating a secretion by active processes In 1964 Hochwald (15) showed that IgA was probably synthesized locally by interstitial cells in the mammary gland later these results were rendered even more probable suggesting the concentration of immunoglobulins to be subject to acute infections of the mammary gland in question (29 30)

In the present material the concentrations of immunoglobulins in milk from the left and the right breast respectively did not differ significantly in the individual woman However all the women in the material were completely healthy without any symptoms of one sided mastitis The relatively high concentration of all the immunoglobulins found in

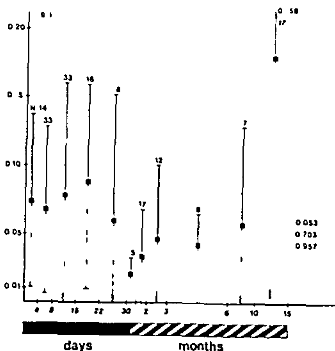


Fig 7 Lysozyme content during period of lactation The material has been divided into groups and the number of samples within each group indicated by *N* In the period 15 to 27 months of lactation lysozyme was found in only 3 samples the values varying considerably

increase in concentration found during the last phase of lactation is due mainly to the decreasing amount of milk as no corresponding increase in the total secretion is registered

IgG is also found in breast milk during the entire lactational period Fig 5 shows the concentration to be high in colostrum but considerably lower than the IgA concentration the mean value being 0 08 g/l ranging from 0 03 g/l to 0 25 g/l Following this the content decreases rapidly and 2 to 3 weeks after birth the secretion is stabilized on the 0 03 g/l level increasing slightly towards the last weeks of the lactational period Also in the case of IgG the individual differences are considerable while the concentration in the individual case follows the pattern outlined above However the total 24 hour secretion of IgG in the individual mother is far more constant right from the start of the lactation, as Table 1 shows

IgM is found in relatively small amounts in breast milk and in determinable quantities only during the first 3 weeks of the lactational period The concentration is also here highest immediately after birth viz 0 1 g/l, ranging from 0 03 g/l to 0 28 g/l as shown in Fig 6 During the following weeks the concentration decreases rapidly After 3 to 4 weeks of lactation only weak traces of IgM can be demonstrated, which condition remains unchanged for the remainder of the lactational period In 3 out of the 4 mothers supplying milk for more than one year after birth, determinable quantities of IgM could be demonstrated in the milk samples viz from 0 02 g/l to 0 04 g/l

The content of lysozyme varied from one day to the next in the individual mother as well as in the material as a whole (Fig 7) No definite correlation was found regarding intervals within the lactational period or regarding the amount of milk secreted as shown in Table 1

The results of the determination of IgA IgG IgM lysozyme and electrolytes in milk from the right and the left breast and in serum appear in Table 2 It should be noted that all serum concentrations were within the normal range for adults for the analytical method in question Analysing all data by the Spearman Ranking Test the following conclusions may be drawn

A significant positive correlation was found between the following concentrations

(1) The concentrations of sodium and chloride ion in milk ($p<0\ 001$ r_s (coefficient of correlation) $= 0\ 90$)

(2) The concentrations of sodium and IgA in milk ($p<0\ 05$ $r_s = 0\ 51$)

(3) The concentrations of IgG in milk and in serum ($p<0\ 01$ $r_s=0\ 74$)

(4) The concentrations of lysozyme in m and in serum ($p<0\ 05$ $r_s = 0\ 54$)

However it was not possible to demc strate any definite relation between the in vidual immunoglobulins and electrolytes in t remaining cases It should be noted that t concentration of IgM was below 5 mg/l some of the milk samples and a statistic analysis of these tests was thus not possible

In all the tests the concentrations

able 2 *Content in milk from right and left mammary gland and in serum on the same day (19 fferent persons)*

nght l=left s=serum

Pat no	Potassium ion (mmol/l)			Sodium ion (mmol/l)			Chloride ion (mmol/l)			IgA (arb U/l)			IgG (g/l)			Lysozyme (mg/l)		
	r	l	s	r	l	s	r	l	s	r	l	s	r	l	s	r	l	s
8	17 6	18 6	4 1	13 0	10 2	136	17	17	109	0 663	0 667	1 72	0 024	0 023	10 08	24 8	25 0	2 14
9	12 4	15 3	4 6	7 4	15 3	139	10	16	102	0 267	0 372	2 77	0 018	0 075	13 38	24 3	35 0	2 19
11	12 8	16 3	4 3	12 5	11 3	137	17	17	103	0 458	0 499	1 63	0 028	0 012	10 39	190 0	217 0	2 06
12	14 7	16 5	4 0	12 8	10 0	138	16	14	100	0 278	0 317	3 80	0 046	0 042	13 51	18 1	13 3	1 80
35	15 1	14 4	3 5	6 3	6 2	122	10	10	102	0 228	0 208	2 52	0 021	0 022	10 07	41 0	38 8	1 54
0	16 7	16 7	4 4	2 7	2 5	138	7	8	93	0 203	0 208	2 72	0 029	0 029	10 41	32 0	32 8	1 82
22	14 3	15 7	4 5	6 0	5 2	136	12	13	97	0 232	0 276	2 30	0 032	0 027	12 34	160 0	170 0	1 90
5	15 7	16 8	3 4	12 9	10 5	138	19	17	105	0 292	0 289	0 80	0 004	0 005	6 25	9 0	7 9	1 82
28	15 1	15 0	4 0	7 7	8 1	139	10	10	102	0 363	0 352	1 29	0.008	0 015	9 90	13 6	16 5	1 79
30	15 7	15 2	4 0	8 2	11 6	140	12	14	100	0 264	0 287	2 96	0 058	0 071	14 29	216 0	202 0	2 68
18	14 3	15 6	4 7	34 0	23 0	140	31	24	86	0 593	0 555	2 78	0 045	0 079	10 70	40 4	37 2	2 70
48	14 0	13 8	5 1	16 0	22 0	144	20	27	107	0 363	0 433	1 63	0 090	0 102	13 61	35 6	36 2	2 10
32	16 7	17 9	4 4	9 0	10 0	143	16	17	104	0 546	0 669	2 50	0 025	0 038	9 43	23 5	27 2	1 70
47	14 7	16 7	4 9	5 0	7 0	144	10	14	107	0 199	0 384	2 00	0 016	0 032	10 68	15 8	34 2	1 10
29	15 9	15 3	4 5	13 0	15 0	139	16	17	106	0 987	0 932	3 48	0 051	0 051	11 62	52 6	63 1	0 93
39	14 4	15 2	4 6	11 0	11 0	141	16	16	103	0 124	0 180	0 85	0 017	0 024	7 77	223 9	245 8	2 28
45	19 3	19 3	4 3	9 0	10 0	142	16	16	106	0 310	0 328	1 15	0 026	0 025	9 40	30 0	28 7	1 71
46	11 0	11 7	4 2	2 0	3 0	143	5	7	104	0 108	0 158	1.00	0 018	0 016	11 84	29 1	37 2	1 19
35	15 9	18 5	4 4	5 0	5 0	140	10	13	102	0 279	0 365	3 50	0 054	0 046	15 83	17 2	13 4	1 10

potassium and lysozyme were much higher in the milk samples than in the corresponding serum samples while the concentration of the other substances was considerably lower in milk than in serum in the individual person

The 24 hour variations of IgA IgG and IgM as well as lysozyme in the milk collected from one of the women appear in Table 3 No significant change in the content of the different immunoglobulins and lysozyme was found either in the course of a single breast feeding or for the 24 hours as a whole

DISCUSSION

The content of the immunoglobulins IgA IgG and IgM is in short high in colostrum decreasing rapidly during the first weeks of the lactational period Three to 4 weeks after birth only weak traces of IgM persisted while IgG and especially IgA were found in determinable quantities during the whole period of lactation e s period lasted for more than 2 y

The primary cause of the individual variations and of the varying level of the concentration curves is probably an active secretion of certain immunoglobulins by the mammary gland (24 27 28 29 30 32) IgA globulin is found in a high concentration in various secretions for instance bile saliva bronchial and nasal secretion and spinal fluid indicating a secretion by active processes In 1964 Hochwald (15) showed that IgA was probably synthesized locally by interstitial cells in the mammary gland later these results were rendered even more probable suggesting the concentration of immunoglobulins to be subject to acute infections of the mammary gland in question (29 30)

In the present material the concentrations of immunoglobulins in milk from the left and the right breast respectively did not differ significantly in the individual woman However all the women in the material were completely healthy without any symptoms of one sided mastitis The relatively high concentration of all the immunoglobulins found in

Table 3 *Lactational phase and variations in 24 hour period in one mother (mean values for 5 24 hour periods) IgA is given in arbitrary units*

Lactational phase	IgA (arb mU/l)	IgG (mg/l)	IgM (mg/l)	Lysozyme (mg/l)
Morning				
start	203	28	54	146
half way	221	29	60	152
end	200	28	55	154
Noon				
start	220	32	57	162
half way	219	29	59	149
end	204	28	50	156
Night				
start	267	30	62	157
half way	285	32	64	174
end	215	30	60	163

milk from the mothers lactating for a considerable length of time i e for more than 9 months after birth is peculiar This has not been demonstrated by other authors no investigations of the various immunoglobulins exceeding the 6th month of lactation Total protein has as mentioned above been determined for at least 24 months after birth (31) Animal experiments have not afforded corresponding results either, but attention was focused in these cases too on the first months of lactation The cause of the phenomenon is uncertain but the following may be a possibility Prolonged lactation and high immunoglobulin concentration may be parallel phenomena due to specific properties of the cells in the individual woman or to external stimuli The rise in the immunoglobulin concentration may also be related to prolonged lactation As only a very limited number of mothers have a lactational period exceeding 9 months and as it is difficult at the commencement of lactation to predict which of the mothers will be able to produce milk for so long a period it will be difficult to solve the problems along these lines

No significant changes could be demonstrated with regard to the 24 hour and to the lactational phase variations either in the case of the individual lactation or for the 24 hour period as a whole This is in complete agreement with the results of other authors (1)

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REFERENCES

1 *Ahlstedt S* & Carlsson B Studies of antibodies in human milk *Naringsforskning 17* Suppl 8 1973
2 Amman A J & Stiehm E R Immune globulin levels in colostrum and breast milk and serum from formula and breast fed newborns *Proc Soc Exp Biol Med 122* 1098 1966
3 Blanc B *Les proteines in lactaeum* Thesis University of Lausanne Editions Medicine et Hygiene Geneva 1964
4 Chodirker W B & Tomasi Jr T B Gammaglobulins quantitative relationships in human serum and nonvascular fluids *Science 142* 1080 1963
5 Documenta Geigy *Scientific Tables (e d) 7* 1970 p 683
6 Documenta Geigy *Scientific Tables (e d) 7* 1970 p 687
7 Gugler E Bokelmann G Datwyler A & von Muralt G Uber Immunoelektrophoretische Untersuchungen an Frauenmilchproteinen *Schweiz Med Wschr 88* 1264 1958
8 Gugler E & von Muralt G Immunoelektrophoretische Untersuchungen an Frauenmilchproteinen II *Schweiz Med Wschr 89* 925 1959
9 Hanson L Å & Johansson B G Immunological investigation of human milk and human milk protein fractions *Int Arch Allergy Appl Immunol 15* 257 1959
10 Hanson L Å Comparative analysis of human milk and human blood plasma by means of diffusion in gel methods *Experientia 15* 473 1959
11 – Immunological analysis of human milk *Int Arch Allergy Appl Immunol 15* 245 1959
12 – The serological relationship between human milk and blood plasma *Int Arch Allergy Appl Immunol 17* 45 1960
13 – Comparative immunological studies of the immune globulins of human milk and blood serum *Int Arch Allergy Appl Immunol 18* 241 1961
14 – *Immunological studies of human milk with special reference to the immune globulins* Thesis University of Gothenburg Gothenburg 1961
15 Hochwald G M Jacobsen E B & Thorbecke G J C Amino Acid incorporation into transferrin and beta 2A globulin by ectodermal glands in vitro *Fed Proc 23* 557 1964
16 Jensen K B Immunochemical determination of serum concentrations of albumin IgG and IgM *Protides Biol Fluids* (Elsevier Amsterdam) *14* 677 1966

17 Laurell C B Quantitative estimation of proteins by electrophoresis in agarose gel containing antibodies *Anal Biochem 15* 45 1966
18 Lubin B Boesman M J Michaels R H Kenney J F & Gitlin D A comparison of the γ globulins of human breast milk and serum *J Pediatr 65* 1103 1965
19 Macy I G Composition of human colostrum and milk *Am J Dis Child 78* 589 1949
0 Mancini G Vaerman J P Carbonara A O & Heremans J F A single radial-diffusion method for the immunological quantitation of proteins *Protides Biol Fluids 11* 370 1964
?1 Mellander O & Vahlquist B Comparative biochemistry of human and cows milk *Acta Paediatr Scand 31* Suppl 116 1959
22 von Muralt G La maturation de l immunité humorale chez l homme *Helv Med Acta 29* Suppl 42 I 1962
?3 von Muralt G Gugler E & Roulet D L A Immunoelectrophoretic analysis *In* Grabar P & Burtins P (eds) *Application to Human Biological Fluids* Elsevier Amsterdam 1964 p ?61
?4 Sabin, A B & Fieldsteel A H Antipoliomyelitic activity of human and bovine colostrum and milk *Pediatrics 29* 105 196?
?5 Schwick G Esser H O & Koch F Qualitative und quantitative immunologische Bestimmung von Plasmaproteinen in Frauenmilch *Behringswerk Mitteilungen* Heft 37 1959 p 11
?6 Thomsen J Lund E H Kristiansen K Brunfeldt & Malmquist J A val val sequence found in a human monocytic leucemia lysozyme *FEBS Lett 22* 34 197? Amsterdam
27 Tomasi jr T B & Zigelbaum S The selective occurrence of γ1 A globulins in certain body fluids *J Clin Invest 42* 1552 1963
?8 Tomasi jr T B Tan E M Solomo A & Prendergast R A Characteristics of an Immune System common to certain external secretions *J Exp Med 121* 101 1965
?9 Tomasi jr T B & Bienenstock J Secretory Immunoglobulins *Adv Immunol 9* 1 1968
30 Tourville D R Adler R H Bienenstock J & Tomasi jr T B The human secretory immunoglobulin system Immunohistological localization of γA secretory piece and lactoferrin in normal human tissues *J Exp Med 129* 411 1969
31 Underwood B A Hepner R & Abdullah H Protein lipid and fatty acids of human milk from Pakistani women during prolonged periods of lactation *Am J Clin Nutr 23* (4) 400 1970
32 Warren R J Lepow M I Bartsch G E & Robbins F C The relationship of maternal antibody breast feeding and age to the susceptibility of newborn infants to infection with attenuated poliovirus *Peidiatrics 34* 4 1964

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2 Amman A J & Stiehm E R Immune globulin levels in colostrum and breast milk and serum from formula and breast fed newborns *Proc Soc Exp Biol Med 122* 1098 1966
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7 Gugler E Bokelmann G Datwyler A & von Muralt G Uber Immunoelektrophoretische Untersuchungen an Frauenmilchproteinen *Schweiz Med Wschr 88* 1264 1958
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15 Hochwald G M Jacobsen E B & Thorbecke G J C Amino Acid incorporation into transferrin and beta 2A globulin by ectodermal glands in vitro *Fed Proc 23* 557 1964
16 Jensen K B Immunochemical determination of serum concentrations of albumin IgG and IgM *Protides Biol Fluids* (Elsevier Amsterdam) *14* 677 1966

ıble I *The treatment trial in 91 girls with 3U but without parenchymal reduction or flux*

ıe bacteriuria eliminated by	
nitrofurantoin	7
a bladder wa hout	16
ɔt treated	68
ɔtal	91
ıe non treated patients during the first months (68)	
he bacteriuria eliminated by	
penicillin	2
spontaneously	4
ost to follow-up	1
ot treated	61
otal	68
ıe non-treated patients randomi ed after months (61)	
on-treatment group	31
reatment group	30
otal	61

In the first 60 of the 116 ABU patients a bladder wash ut *test* was performed as earlier described (4 11) and a ıine sample was also *obtained one day after the test* The ırls with parenchymal reduction were treated at once *even* of the 13 patients with reflux but without arenchymal *reduction were* observed without treatment ɔr 6 months after which they were treated like the *remain* ıg 6 patients Seven of the 91 girls without parenchymal eduction *or reflux* were treated at once one since she had bladder diverticulum and 6 because of *enuresis or nasty* melling urine The rest of the 91 patients were observed ɔr *6 months* without treatment and 61 subjects still acteriuric after the *observation period* were randomly ıssigned to treatment and no treatment groups (Table I)

Diagnostic procedures and criteria Midstream urine amples were kept at +4°C *until* semi-quantitatively ultured (8) To obtain a second sample in patients with a uspected asymptomatic recurrence the patient was in structed to collect *morning* midstream urine and to culture t with a dipslide Uricult® (Orion Pharmaceutical Co Helsinki Finland) at home The slide was sent by mail to he laboratory and read after incubation over night at 37°C Classification of the bacteria and *0-grouping of E coli* was performed as earlier described (8) The con entrating capacity of the kidneys was estimated by freeze point reduction in two consecutive *urine samples obtained* at home after fluid deprivation for at least 15 hours The samples with the highest osmolality was noted The age related normal values by Winberg (16) *were used* as a reference If the concentrating capacity was <814 mOsm/l the test was repeated Blood samples were obtained and analyzed for *microsedimentation rate* (ESR) and C-reactive protein (CRP) (13)

A diagnosis of an a ymptomatic recurrence was made if the patient had no obvious symptoms but two urine sam ples with ≥100 000 *bacteria/ml urine* and the same type of bacteria in both cultures The criteria for symptomatic cystitis were acute frequency and burning temperature not exceeding 38 0°C significant bacteriuria as well as *normal renal concentrating* capacity (16) and for pyelonephritis fever >38 0°C a bacterial count of ≥100 000 bacteria/ml urine and at least two positive of the tests *ESR >20 mm/h CRP>10 µg/ml or renal* concentrat ing capacity<814 mOsm/l (4) A spontaneous remission was diagnosed if two negative urine cultures were found on at least two successive examinations

Therapy Patients *with ABU* selected for the treatment group and patients with symptomatic cystitis were given nitrofurantoin 3 mg per kg per 24 hours for 10 days Long term prof)laxis 6 months with nitrofurantoin 1 mg per kg per 24 *hours was given after initial sterilization of* urine to patients recurring after a second short treatment course Patients with symptomatic pyelonephritis were given sulfisoxazole (Gantrisin®) 200 mg per kg per 24 hours for 10 days

Follow-up The patients were examined every third or sixth month when urine for culture blood samples for CRP and *in symptomatic patients ESR were obtained* The renal concentrating capacity was tested Urine for culture was also always obtained within a week after com pletion of antibiotic therapy

For *statistical* evaluation the Wilcoxon test *for pair* differences was employed

RESULTS

The 12 patients with parenchymal reduction (11/12 with reflux)

One patient had her bacteriuria eliminated and did *not* recur after a bladder washout test Eleven patients had a negative culture after the initial *short term* treatment with nitro-furantoin and 6 of them did not recur for 1½ years Three girls had a symptomatic pyelonephritis 3 6 and 13 months respectively after the initial treatment One girl got a symp tomatic cystitis 13 months after the initial treatment and one patient had two asymp tomatic recurrences after 16 months

The mean renal concentrating capacity be fore treatment 869±124 mOsm/l (±1 S D) increased significantly ($p<0.01$) to 984±77 mOsm/l (±1 S D) one year after the initial *treatment By* this time all patients had been abacteriuric for at least 6 months (Fig 1) In creased serum CRP *found in* 4 patients and ESR>20 mm/h found in 2 *patients* at detection

Acta Pædiatr Scand 64 718–724, 1975

ASYMPTOMATIC BACTERIURIA IN SCHOOL GIRLS

V The Clinical Course and Response to Treatment

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ABSTRACT Lindberg U (Department of Paediatrics and Department of Immunology, Institute of Medical Microbiology, University of Goteborg Goteborg Sweden) Asymptomatic bacteriuria in school girls V The clinical course and response to treatment Acta Paediatr Scand, 64 718, 1975 —The short term prognosis of 116 school girls with asymptomatic bacteriuria (ABU) treated or left untreated is reported In untreated girls a spontaneous remission was obtained in 11% within one year A short course of nitrofurantoin eliminated the bacteriuria in 93% of the girls in the treatment group The recurrence rate was 30% in one year in the patients that became abacteriuric spontaneously, after a bladder washout test or after treatment with penicillin or nitrofurantoin The first recurrence occurred within 3 months in 79% (19/24) Nineteen of the 24 patients with recurrences (79%) had a third infection within 9 months In patients with parenchymal reduction or reflux the renal concentrating capacity increased significantly after treatment of the bacteriuria All the girls left untreated had normal renal concentrating capacity on detection and this remained unchanged during the year of follow up One of 28 untreated girls attracted a symptomatic pyelonephritis caused by a strain different from that at detection of the ABU Of 81 girls cured from the bacteriuria 24 recurred, 5 with a symptomatic pyelonephritis and 3 with cystitis It is concluded that strains isolated from girls with ABU do not commonly cause symptomatic pyelonephritis

KEY WORDS Asymptomatic bacteriuria school girls treatment non treatment short term prognosis

Since the long term consequences of asymptomatic bacteriuria (ABU) in children are uncertain and its association with renal infection is unknown, a follow up of school girls with ABU was started in Goteborg 1971 The clinical and laboratory findings at detection have been reported and it was found that 10% of the girls had parenchymal reduction on the pyelogram (10) which is in agreement with Kunin et al (6) Whether this kidney damage is a result of earlier symptomatic infections or silent bacteriuria is not known

The study was supported by grants from the First of May Flower Campaign the Faculty of Medicine University of Goteborg and from the Insurance Company Forenade Liv Sweden

A follow up of untreated patients with ABU will add to the information about the natural history of this disease The present study is concerned with the clinical course of ABU treated or untreated and followed for 1–1½ years in the earlier described group of pat (9 10 11 12)

MATERIAL AND METHODS

The material comprised 116 school girls 7–15 yea with ABU detected during screening (10) They grouped according to roentgenological findings (1 patients with parenchymal reduction (11/12 also wi flux) 13 girls with reflux but without parenchymal r tion and 91 patients with neither parenchymal redu nor reflux

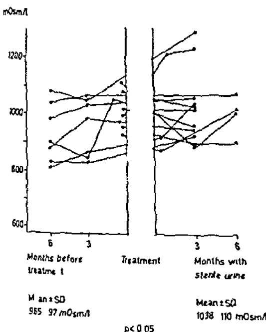


Fig 2 The renal concentrating capacity before and after treatment in 13 girls with ABU and reflux but without parenchymal reduction

nated their bacteria Of the remaining 75 girls 6 were given nitrofurantoin for nasty smelling urine or for enuresis and one for a bladder diverticulum Two of these 7 girls attracted symptomatic pyelonephritis one month after the initial treatment and one got a cystitis after 7 months Two of the 7 patients got an asymptomatic recurrence within one month and 2 have not recurred for 1½ years

Of the 68 girls (all with *E coli* in their urine) followed untreated for 6 months 2 became abacteriuric after they had been given penicillin for otitis and tonsillitis and 4 became spontaneously abacteriuric and have had no recurrence for 1½ years One girl was lost to follow up because she moved from the area The 61 patients still bacteriuric and asymptomatic during 6 months of observation were randomized into treatment and nontreatment groups

In 6 girls with increased CRP values at the detection (10) no CRP was detectable after they cleared their bacteriuria one spontaneously 2 after treatment with nitrofurantoin and 3 after a bladder washout ESR>20 mm/h found in 2 girls was normalized in one girl without treatment of the bacteriuria The other had a normal value after a bladder washout had cured the bacteriuria

Treatment group 30 patients All patients treated with a short course of nitrofurantoin became abacteriuric except 2 in whose urine the same serological type of *E coli* was found before and after the initial treatment After another short time treatment with nitrofurantoin the bacteriuria was eliminated Three of the 30 girls moved from the area during the observation period Fifteen of the remaining 27 patients (56%) had no recurrence for one year The other 12 (44%) recurred twice or more In 9 the first recurrence occurred in 3 months in one after 3–6 months and in 2 after 6–9 months One of the recurring girls had a symptomatic pyelonephritis 2 months after the second short time treatment

The mean concentrating capacity of the kidneys did not differ significantly before and after treatment 1 030±103 mOsm/l (±1 S D) and 1 019±76 mOsm/l respectively

The untreated group 31 patients One patient moved from the area during the observation period Two patients became abacteriuric after treatment with penicillin for respiratory tract infection and they have not recurred Three patients became spontaneously abacteriuric and have not recurred One girl had an attack of symptomatic pyelonephritis after 15 months of observation The urine culture then disclosed an *E coli* strain similar to the earlier found but also significant numbers of *Enterococci*

After 18 months of observation there were 24 girls still with ABU They all had the same serotype of *E coli* throughout the observation period except one in whose urine spontaneously—nonspecifically—agglutinating *E coli* were isolated after a follow up of 12 months

The mean concentrating capacity of the kidneys in the bacteriuric girls at 6 and 12 months were not different from the value

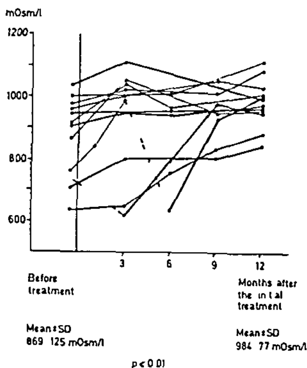


Fig 1 The renal concentrating capacity before and after treatment in 12 girls with ABU and parenchymal reduction (11/12 with reflux) The broken lines indicate the decrease in concentrating capacity during recurrence with acute pyelonephritis

(10) had normalized after treatment with nitrofurantoin

The 13 patients with reflux but without parenchymal reduction

One of the 13 girls became abacteriuric after a bladder washout test and did not recur during the one year follow up Seven of the remaining 12 girls treated with a short course of nitrof rantoin did not recur, while 5 recurred twice more during the first year In 2 girls the recu rences were combined with cystitis sympton and in the other 3 they were asymptomat (Table 2) *E coli* of the same serological ty and sensitive to nitrofurantoin was found b fore and after the initial treatment in 2 of th recurring patients After another short tim treatment with nitrofurantoin there was a immediate cure of the bacteriuria The firs recurrence occurred within 3 months after th initial treatment

Seven of the treated patients were observe for 6 months before treatment *During th* period there was no decrease of the renal con centrating capacity (Fig 2) The mean rena concentrating capacity of all 13 patients before treatment 965±97 mOsm/l (± 1 S D) in creased significantly ($p<0.05$) to 1038±11C mOsm/l (± 1 S D) when the patients had been abacteriuric for 3–6 months (Fig 2) Elevated CRP found in one girl at detection (10) normalized spontaneously during the observa tion period without treatment while the in creased *ESR found in one girl* was normal after treatment with nitrofurantoin

The 91 patients without parenchymal reduction or reflux (Table 1)

Sixteen patients did not recur for 1½ year after an initial bladder washout test which elimi

Table 2 *Recurrence rate during one year after an immediate cure in 81 girls with asymptomatic bacteriuria*

Each patient with a recurrence is represented only once

Roentgenological changes	No of patients	No recurrence	Asymptomatic recurrence	Symptomatic cystitis	Symptomatic pyelonephritis
Parenchymal reduction (11/12 with reflux)	12	10	0	0	2
Reflux without parenchymal reduction	13	8	3	2	0
No reflux nor parenchymal reduction	56	39	13	1	3
Total	81	57 (70%)	16 (20%)	3 (4%)	5 (6%)

elonephritis or cystitis appearing after cure the ABU were caused by *E coli* strains of an group different from the one present before atment and in contrast to the strains causing ABU they were more resistant to the ctericidal effect of normal serum (7) The treated girls however had the same rotype of *E coli* during the observation riod and these strains did not cause symp matic pyelonephritis or cystitis This supports the observation discussed in an earlier aper that ABU patients are infected with trains which differ from those causing symp omatic infections (12)

Neither seemed the bacteria isolated from he ABU patients to involve the kidneys during the observation period in the untreated girls without parenchymal reduction or reflux No increase in serum CRP was found and when the renal concentrating capacity was followed there was no decrease When followed in the treated girls without roentgenological changes there was no difference before and after treatment However in the girls with parenchymal reduction and reflux and in those with reflux but without parenchymal reduction there was a significant increase in the concentrating capacity of the kidneys after treatment This was also found by Savage et al in 5 year-old girls with ABU (15) Since in the present study micturition cystographies have not been performed when the urine became sterile it is impossible to evaluate the role of the reflux for the decrease of the renal concentrating capacity

It is concluded that ABU in schoolgirls is probably not the forerunner of symptomatic pyelonephritis Such infections seem to be caused by strains which differ from those ound in ABU The follow up of renal concentrating capacity in untreated girls with ABU without any renal parenchymal reduction or reflux gave no indication of renal involvement Detectable structural abnormalities in the urinary tracts will be eval uat

REFERENCES

1 Asscher A W Sussman M Waters W E Evans Joy A S Campbell H Evans K T & Edmund Williams J Asymptomatic bacteriuria in the non pregnant woman II Response to treatment and follow up *Br Med J I* 804 1969
2 Bergstrom T Lincoln K Redin B & Winberg J Studies of urinary tract infections in infancy and childhood X Short or long term treatment in girls with first or second-time urinary tract infections uncomplicated by obstructive urological abnormalities *Acta Paediatr Scand 57* 186 1968
3 Cox C E & Hinman F Factors in resistance to infection in the bladder I The eradication of bacteria by vesical emptying and intrinsic defense mechanisms *In* E H Kass (ed) *Progress in Pyelonephritis* F A Davis Co Philadelphia 1965 p 563
4 Jodal U Lincoln K & Lindberg U Level diagnosis of symptomatic urinary tract infections in childhood *Acta Paediatr Scand 64* 201 1975
5 Kass E H Savage W & Santamarina B A G The significance of bacteriuria in preventive medicine *In* E H Kass (ed) *Progress in Pyelonephritis* F A Davis Co Philadelphia 1965 p 3
6 Kunin C M Deutscher R & Paquin A Jr Urinary tract infections in school children An epidemiologic clinical and laboratory study *Medicine 43* 91 1964
7 Lidin Janson G Hanson L Å Lincoln K Lindberg U & Olling S Asymptomatic bacteriuria in school girls VI Differences in *Escherichia coli* causing symptomatic and asymptomatic recurrence To be published
8 Lincoln K Lidin Janson G & Winberg J Resistant urinary infections resulting from changes in resistance pattern of faecal flora induced by sulphonamide and hospital environment *Br Med J III* 305 1970
9 Lindberg U Bjure J Haugstvedt S & Jodal U Asymptomatic bacteriuria in school girl III Relation between residual urine volume and recurrence *Acta Paediatr Scand 64* 437 1975
10 Lindberg U Claesson I Hansson L Å & Jodal U Asymptomatic bacteriuria in school girls I Clinical and laboratory findings *Acta Paediatr Scand 64* 425 1975
11 Lindberg U Hanson L Å Jodal U & Kaijser B Asymptomatic bacteriuria in school girls IV Difficulties in level diagnosis and the possible relation to the character of infecting bacteria *Acta Paediatr Scand 64* 574 1975
12 Lindberg U Hanson L Å Jodal U Lidin Janson G Lincoln K & Olling S Asymptomatic bacteriuria in school girls II Differences in *Escherichia coli* causing asymptomatic and symptomatic bacteriuria *Acta Paediatr Scand 64* 432 1975
13 Nilsson L Å Comparative testing of precipitation methods for quantitation of C reactive protein in blood serum *Acta Pathol Microbiol Scand 73* 1 9 1968

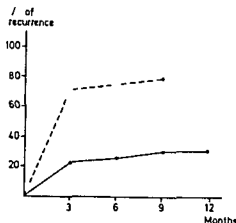


Fig 3 The cumulative rate of recurrence within one year after the first cure in 81 girls with ABU (—) and within nine months after the second cure in 24 girls ( )

found when the initial diagnosis of asymptomatic bacteriuria was made 1 039±110 mOsm/l (±1 S D), 1 016±92 mOsm/l and 1 004±106 mOsm/l respectively No increase in serum CRP was found in the girls when asymptomatic

The recurrence rate in the 81 girls cured from their bacteriuria The number of patients recurring (30%) among 81 girls i e the 116 patients minus the 31 girls not treated and the 4 which were lost to follow up are listed in Table 2 These 81 patients became abacteriuric spontaneously after a bladder washout test or after treatment with penicillin or nitrofurantoin There was no difference in recurrence rate between patients with parenchymal reduction or reflux (7/25 28%) and those without any such radiologic signs (17/56 30%)

The first recurrence in the 81 girls occurred within 3 months in 19 girls (23%) within 6 months in 21 girls (26%) and within 9 months in 24 girls (30%) (Fig 3) The second recurrence in the 24 girls with a first recurrence occurred in 17 patients (71%) within 3 months in 18 (75%) within 6 months and in 19 (79%) within 9 months (Fig 3)

DISCUSSION

The present study provides information about the short term natural history of ABU in school girls After a follow up of only six months 6% of the untreated bacteriuric subjects had undergone a spontaneous remission During the following year when the still bacteriuric patients were randomized into two groups (treated and untreated), a spontaneous remission was obtained in 11% (3/27) of the untreated ones The findings of spontaneous cure is supported by both experimental and epidemiological observations Cox & Hinman (3) showed that *E coli* was cleared from the urinary tract of normal human volunteers within 72 hours and Asscher et al found a spontaneous remission rate of 36% in adult non pregnant women with ABU (1) while Kass et al found a rate of 25% (5) A failure to empty the bladder completely can probably affect the ability of the bacteriuric patients to eliminate the infection (14) In an earlier study of the role of residual urine in girls with ABU it was found that 43% had volumes >5 ml and that recurrences were significantly more common in those with >5 ml residual urine volume than in those with <5 ml It was also found that 4 of the 5 girls tested for residual urine who had undergone a spontaneous remission had residual urine volumes <3 ml (9)

The presence of residual urine may probably increase the risk of recurrences (9) but findings of parenchymal reduction on the pyelogram or reflux on the urethrocystogram did not seem to increase the risk of recurrences during the observation period which was also reported by Kunin et al (6)

The frequency of recurrence found in this study (30%) may be compared with that found by Bergstrom et al in girls 2 months to 16 years old with a first or second known acute symptomatic urinary tract infection (2) Within one year after a short term treatment there was an asymptomatic recurrence in 11% and a symptomatic in 25% As in the present study most of the recurrences occurred within 3 months after the original infection

The clinical presentation of the recurrences seemed to be determined by the character of the infecting strains The symptomatic

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RENAL CONTROL OF SODIUM AND FLUID BALANCE IN NEWBORN INFANTS DURING INTRAVENOUS MAINTENANCE THERAPY

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ABSTRACT Aperia A Broberger O Thodenius K and Zetterstrom R (Department of Paediatrics Karolinska Institutet St Goran s Hospital for Children Stockholm Sweden) Renal control of sodium and fluid balance in newborn infants during intravenous maintenance therapy *Acta Paediatr Scand 64 725 1975*.—*Changes in accumulated* fluid and sodium balance during intravenous maintenance fluid therapy has been studied in 38 newborn infants with different clinical disorders and gestational ages 28–42 weeks The results from the infants born before 36 weeks of gestation (preterms) have been compared with the result from infants born after 36 weeks Three different saline infusions 10 20 and 40 mEq Na/l 1 000 ml 5 5% glucose have been given The infusion rate has in preterm neonates been 3.3 ml/kg and hour and in the more full term neonates been 3 6 ml/kg and hour The study lasted for 5–8 hours Urine was collected by spontaneous voidings in plastic bags The balances were calculated as the difference between the amount given intravenously and the amount excreted in the urine *In the more full-term neonates Na balance became increasingly negative with the 10 mEq* solution Just balanced with the 20 mEq solution and increasingly positive with the 40 mEq solution A different response was found in the preterm neonates. The natriuresis was higher and the sodium balances were increasingly negative with both the 10 and 20 mEq solutions With the 40 mEq solution the negative balance tended to level off The fluid balances were fairly well maintained in all infants regardless of the sodium concentration in the infusate

KEY WORDS Newborn infants preterm sodium balance water balance intravenous maintenance fluid renal function

use of intravenous maintenance therapy ewborn infants has become a more com procedure during the past decades Pre ption of the saline content in the fluid given generally been based on empirical unds since basal data on actual needs of nates are scanty When the saline content he infused fluid has approached 145 mEq/l the content in Ringer solutions oedema mation has been a frequent finding It has refore been assumed that the salt tolerance the neonate is fairly low this assumption has also been supported by the fact that the sodium content of breast milk is low and by the suppressed response to oral salt load characteristics for newborn infants (3 4) When however no extra or very low amounts of sodium has been added to the intravenous fluid hyponatremia has sometimes been recorded

The purpose of this study has therefore been to gain more direct information of the changes in sodium and fluid balance in neonates of different gestational ages who for various clinical reasons have received intravenous maintenance fluid and electrolyte therapy The results from preterm and full term infants have been grouped separately since it has

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14 O Grady F & Cattell W R Kinetics of urinary tract infection II The bladder *Br J Urol 38* 156 1966
15 Savage D C L Wilson M I & Fee W M Renal concentrating ability in 5 year-old girls with covert bacteriuria *Arch Dis Child 47* 14 1972
16 Winberg J Determination of renal concentrating capacity in infants and children without renal disease *Acta Paediatr Scand 48* 318 1959

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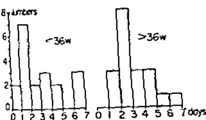


Fig 3 The number of days during which the infants have received intravenous fluid before the study started

of 3 6 and 3 3 ml/kg/hour respectively In 18 of the infants a 10% inulin (Laevasar Gesellschaft) solution was given at the rate of 0 01 ml/kg and minute into the same vein used for the saline infusion The infusion was preceded by a prime dose of 0 5 ml/kg body weight

Urine and blood sampling

Urine was collected by spontaneous voidings in plastic bags Immediately after voiding the urine was withdrawn by a syringe through a tube This method has previously been proved to be valid for urine collections in infants (3 4) The time interval between each voiding was recorded Capillary blood samples were taken by heel puncture during each voiding period Inulin was determined in each of the blood samples Sodium total protein osmolality and hematocrit were generally only analyzed in the blood samples obtained in the beginning and towards the end of the study In 2 infants the glomerular filtration rate was estimated by single injection technique with inulin as an indicator as previously described (3)

Calculations

The time interval for each voiding period was determined and the amount of fluid and Na given during each such period was calculated The salt balance for each period was calculated as the difference between the amount of sodium given intravenously and the urinary Na excretion The accumulated changes in balance during the study were then determined The fluid balance was calculated in the same way The glomerular filtration rate was calculated according to the formula

$$\frac{U \times V}{P}$$

where V represents the diuresis

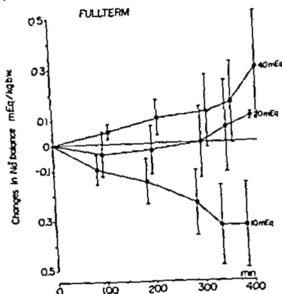


Fig 4 The accumulated changes in sodium balance in infants born after 36 weeks of gestation when receiving the three different saline solutions ● Mean values of sodium balance Range bar represents standard error of mean (S E M)

Analytical methods

The sodium concentrations in serum and urine were analysed by flame photometer (Eppendorf) Inulin in blood and urine was determined with the anthron method (8) Osmolality in blood and urine was determined cryoscopically with the aid of a Knauer osmometer Serum protein was determined refractrometrically Hematocrit was estimated in glass capillaries which were centrifuged at 10 000 rpm for 5 minutes

RESULTS

The changes in sodium balance accomplished by the kidney during intravenous infusion in infants above 36 weeks of gestation are illustrated in Fig 4 It is apparent that when the infusate contained only 10 mEq Na^+/1 000 m

Table 2 *The distribution of the groups and the number of infants studied at the three saline solutions Mean value of gestational age in weeks ± 1 S D are given*

	10 mEq Na		0 mEq Na		40 mEq Na	
	<36 w	>36 w	<36 w	>36 w	<36 w	>36 w
Number of infants	n=8	n=10	n=6	n=5	n=5	n=4
Gestational age (weeks)	3? 0±? 7	39 0±1 7	3 3±? 0	37 6±1 8	3? 0±? 0	41±1 4

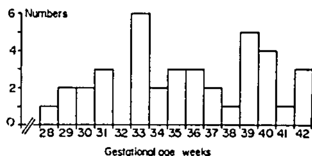


Fig 1 The number and distribution of the infants studied

previously been shown that preterm infants significantly differ from full term infants in the response to an oral salt and fluid load (4)

MATERIAL AND METHODS

Material

Thirty eight newborn infants of gestational ages 28–42 weeks have been studied The gestational age was calculated from the mother s last menstrual period It was then confirmed by the appearance of the external features and the results from the neurological examination (2 4) The distribution of the gestational ages of the infants is demonstrated in Fig 1 The infants have been divided into one group born before 36 weeks of gestational age and one group born after 36 weeks of gestation The postnatal age at the time of study is given in Fig 2 The studies were performed when the infants for therapeutic reasons received intravenous maintenance fluid therapy In Table 1 the disorders of the infants are given Most of the preterm

Table 1 *Clinical disorders The numbers of infants born before and after 36 gestational weeks are given*

Diagnosis	<36	>36
Idiopathic respiratory distress syndrome[a]	10	–
Pulmonary atelectasis	2	9
Asphyxia	–	2
Neonatal septicemia	–	1
Intraabdominal hemorrhage	–	1
Esophageal atresia	1	–
Colonic atresia	–	1
Anal atresia	–	1
Congenital megacolon	–	1
Common mesentary	1	–
Diaphragmatic hernia	–	1
Incarcerated inguinale hernia	–	1
Feeding difficulty	–	1
Preterm birth (uncomplicated)	5	–

[a] Five of the infants were treated in a respirator or else given CPAP and 2 of them subsequently died on the third day after the study

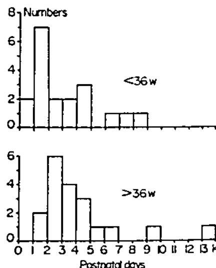


Fig 2 The number and distribution of infants below and above 36 weeks of gestation and their different postnatal age in days at the time of the study

infants had idiopathic respiratory distress syndromes and a few of them were also treated in a respirator or else given continuous positive air pressure (CPAP)

General protocol of study

Prior to the study the infants had received intravenous therapy for least 24 hours except in 2 infants studied on the first day after birth (Fig 3) The intravenous fluid was given through an umbilical vein or artery In a few infants a peripheral vein was used The studies were carried out from September 1972 to September 1974 When this study was started a solution containing 5 5% glucose 10 mEq Na and 20 mEq K /1 000 ml was the routine used for intravenous administration to infants in this hospital Due to the preliminary results from this study the saline content was changed from 10 to 20 mEq/1 000 ml in September 1973 The fluid has been administered as 60 ml/kg during the first day after birth 80 ml/kg during the second day 90 ml/kg during the third day and 95–100 ml/kg and day for the following days The fluid prescription is in accordance with the recommendation given by others (7 9) The studies were started in the morning and generally continued for 5 to 8 hours The studies were performed in the nursery with the infants in their incubators No oral nutrition was given during the study All infants received an infusion of a 5 5% glucose solution containing 20 mEq K /1 000 ml and 10 20 or 40 mEq Na All infants receiving the 10 mEq Na solution had the same solution before the study was started Infants receiving hte 20 mEq solution had received either the 10 or 20 mEq solution before the study Infants receiving the 40 mEq solution had all received the 20 mEq solution before the study was started The distribution of infants receiving the three different saline concentrations in the infusate is demonstrated in Table 2 The solutions were administered by continuous infusions controlled by a slow injector (B Braun Melsingen) The flow rate was tested in each experiment The amount of fluid given averaged in infants above 36 weeks 87 ml/kg and day and in infants below 36 weeks 80 ml/kg and day corresponding to an infusion rate

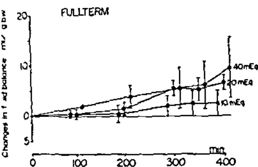


Fig 6 The accumulated changes in fluid balance in infants born after 36 weeks of gestation when the three different saline solutions were given ● Mean values Range bars represent standard error of mean (S E M)

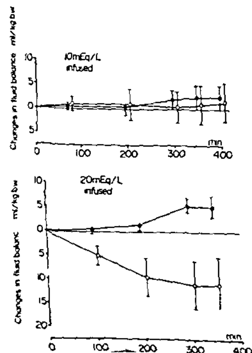
also negative during the entire course of the study but there was no progressive increase of the Na^+ deficit The changes in sodium balance in preterm infants have been compared with those observed in full term infants for each solution studied It is apparent that the Na^+ losses during maintenance therapy were more pronounced in preterm infants than in full-term infants regardless whether the infusate contained 10 20 or 40 mEq Na^+

Fig 6 illustrates the changes in fluid balance induced by changes in urinary water excretion during the intravenous infusion in infants above 36 weeks of gestation The balance was generally slightly positive No statistical difference could be found between the groups of infants receiving the different salt solutions

In Fig 7 *a b c* the changes in fluid balance in infants below 36 weeks of gestation are compared were infants of higher gestational ages It is apparent that in the preterm infants more water is excreted than in full term infants The fluid balances were positive except when *20 mEq Na^+/1 000 ml was given*

The results of the estimation of glomerular filtration rate (GFR) are given in Table 3 The mean values are in all the different groups lower than the GFR values previously found in the neonatal period (4) In Table 3 the average urinary sodium excretion and the average serum sodium values during the study are also given The salt excretion for full term infants is slightly higher than that found in full term healthy infants following an oral salt load (3)

No changes were found in serum protein



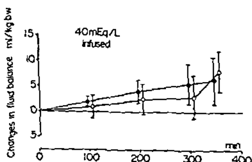
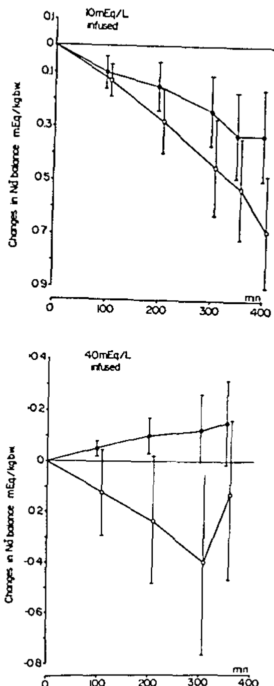


Fig 7 The accumulated changes in fluid balance in infants born before 36 weeks of gestation (O) compared to infants born after 36 weeks of gestation (●) *(a–c) Data* when the three different saline solutions were given Circles represent mean values Range bars represent standard error of mean (S E M)



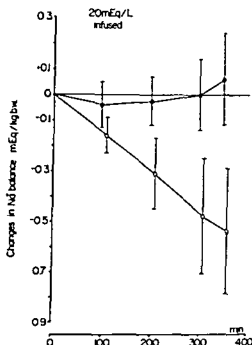


Fig 5 The accumulated changes in sodium balance in infants born before 36 weeks of gestation (unfilled circles) compared to infants born after 36 weeks of gestation (●) (*a–c*) Data from the changes when the different saline solutions were given are demonstrated The circles represent mean values range bars represent standard error of mean (S E M)

the sodium balance became progressively negative i e more sodium was excreted in the urine than given intravenously When however the infusate contained 20 mEq Na^+/1 000 ml the infused amount was almost balanced by the urinary losses At 40 mEq Na^+/1 000 ml the sodium balance was increasingly positive Due to the heterogeneity of the material the individual variations were however fairly large The individual differences were not however due to the different postnatal days The limited number of study did not allow any correlation between the clinical picture and the natriuresis The difference in Na^+ balance when the 10 mEq and 40 mEq Na^+ solutions were given were however almost significant at 350 and 400 minutes (0 $1>p>0$ 5)

In infants below 36 weeks of gestation the sodium balance became negative with the three saline solutions used in this study (Fig 5 *a b c*) Both when 10 and 20 mEq Na^+/1 000 ml were given the sodium balance became increasingly negative When 40 mEq Na^+/1 000 ml was given the average Na^+ balance was

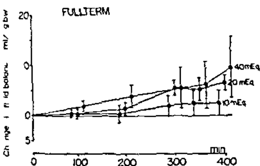


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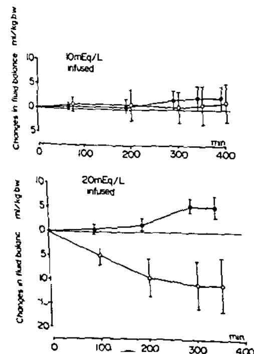
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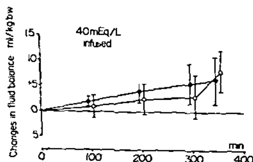


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Tible 3 *The average urinary sodium excretion (UNaV) serum sodium (SeNa) and glomerular filtration rate (GFR) in all studies The values are given as mean ± S E M*

Different saline solutions in mEq Na^+/1 000 ml	10		20		40	
Gestational (weeks)	<36	>36	<36	>36	<36	>36
UNaV mEq/hour/1 73 m^2	[illegible]±1 64	2 52±0 86	3 37±0 85	1 91±0 62	5 1±2 2	1 8[illegible]±0 5
SeNa mEq/l	135 6±2 4	135 3±1 8	138 8±1 2	140 4±2 4	136 8±2 1	139 0±[illegible] 1
GFR ml/min/1 73 m^2	12 7±4 2	19 2±6 3	14 7±2 8	17 6±4 4	–	–

hematocrit and osmolality during the studies The hematocrit values were however, somewhat lower, mean value 54% than in previous studies on healthy full term and preterm neonates (3 4)

DISCUSSION

There does not seem to be any general agreement about what should be the optimal Na^+ intake for the newborn infant As a consequence the Na^+ content in fluid used for intravenous maintenance therapy to newborn infants varies much (1 6 7 11 12) In Sweden the Na^+ content has ranged from 0–150 mEq/1 000 ml The main reason for the uncertainty in prescribing Na^+ to neonates has been lack of basal data on actual requirement and on renal excretion We therefore found it necessary to make a direct study on the maintenance of Na^+ and fluid balance during intravenous administration before recommendations of the optimal Na^+ concentration in the infusate could be given

It is apparent that the kidney of the newborn infant has a very low sodium tolerance both with regard to enhanced excretion during sodium loading and with regard to retention during sodium depletion When newborn full term infants were given 10 20 or 40 mEq Na^+ solutions there was no significant difference in the urinary sodium excretion When only the 10 mEq Na^+ solution was given the neonates were unable to increase the sodium reabsorption despite an increasingly negative balance It is somewhat puzzling why full term infants should not maintain a positive salt balance when the infusate contains 10 mEq Na^+/l since breast milk contains only 7 mEq Na^+/l It should be noticed that in the present study the sodium balance was calculated as the difference between the amount given intravenously and the amount excreted in the urine Since the sodium losses in sweat and stool were neglected the actual sodium balance must in fact be more negative than the calculated sodium balance The question therefore arises whether the infants will respond differently to oral and intravenous administration of sodium In such a case an unexpected result could be explained by a descrepancy in the relationship between net sodium and net water reabsorption from the intestine Other factors could however also contribute to the unexpected finding Since several of the infants included in this study had respiratory problems the present material might not be comparable to normal healthy infants It has in fact been shown that infants with respiratory hypoxia have a significantly increased urinary sodium excretion despite a fairly low glomerular filtration rate (10 13) It is noteworthy that the full term infants in the present study excreted more sodium than what has been recorded previously in healthy newborn infants (3) This emphasizes that recordings made in normal healthy infants cannot be directly applied when evaluating the fluid and electrolyte balance of the sick infant

The preterm infants were found to differ significantly from the full term infants with regard to the ability to retain sodium Urinary

sodium excretion was higher in the preterm infants with all the three saline solutions given As a result the accumulated changes in sodium balance became more pronounced negative for each saline solution given Only when the 40 mEq solution was given the preterm infants could during 6–7 hours of observations barely maintain a slightly negative sodium balance When a 20 mEq solution was given the sodium balance became increasingly negative The exaggerated urinary sodium excretion in preterm infants has previously been observed during oral salt loading (4) Increased sodium losses during first week of life in infants of less than 35 weeks gestation have also been demonstrated at oral feeding (5)

Since the neonates are in a period of rapid growth a slightly positive sodium and fluid balance should be necessary The amounts of fluid given seem to be adequate since the fluid balance remained fairly well maintained regardless of the sodium concentration in the infusate Since the 10 mEq Na^+ solution resulted in an increasingly negative balance for all infants the continuous administration of this solution should eventually result in hyponatremia In our experience this is not an unusual clinical finding when preterm infants have received intravenous therapy for a longer period of time Thus on the basis of the present dindings it seems justified to recommend at least 20 mEq Na /1 000 ml to full term infants and 40 mEq Na /1 000 ml to preterm infants that receive the major part of their fluid intake parenterally for more than one day

REFERENCES

1 Abrahamson H Resuscitation of the newborn infant p 40 C V Mosby St Louis 1973
Amiel Tison C Neurological evaluation of the maturity of newborn infants Arch Dis Child 43 89 1968
3 Aperia A Broberger O Thodenius K & Zetterstrom R Renal response to an oral sodium load in newborn full-term infants Acta Paediatr Scand 61 670 1972
4 — Developmental study of the renal response to an oral salt load in preterm infants Acta Paediatr Scand 63 517 1974
5 Butterfield J Lubchenco L Bergstedt J & O Brien D Patterns in electrolyte and nitrogen balance in the newborn premature infants Pediatrics 26 777 1960
6 Calcagno P L & Hollerman C E Parenteral and electrolyte fluid therapy Current pediatric therapy p 793 W B Saunders Philadelphia London Toronto 1973
7 Davies P A Robinson R J Scopes J W Tizard J P M & Wigglesworth J S Medical care of newborn babies p 99 William Heinemann Medical Books Ltd London 1972
8 Hilger H H Klumper J D & Ullrich K J Wasserrucksresorption und Ionentransport durch die Sammelrohrzellen der Saugetierniere Arch Gesamte Physiol 267 218 1958
9 Holliday M A & Segar W E The maintenance need for water in parenteral fluid therapy Pediatrics 19 823 1957
10 Nicolopoulos D M & Smith C A Metabolic aspects of idiopathic respiratory distress (hyaline membrane syndrome) in newborn infants Pediatrics 28 706 1961
11 Schaffer A J & Avery M E Diseases of the newborn p 851 Saunders Philadelphia London Toronto 1971
12 Sinclair J C Driscoll J M Heird W C & Winters R W Supportive management of the sick neonate Parenteral calories water and electrolytes Pediatr Clin North Am 17 863 1970
13 Torrado A Renal function in idiopathic respiratory distress syndrome Third Int Symp Pediatr Nephr Washington USA 1974
14 Usher R McLean F & Scott K Judgement of fetal age II Clinical significance of gestational age and an objective method for its assessment Pediatr Clin North Am 13 835 1966

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The preterm infants were found to differ significantly from the full term infants with regard to the ability to retain sodium Urinary

ble 1 *Details of infants fed intravenously*

	Age at onset Diarrhoea	Age at start IVF	Total days IVF	Comments
Haemorrhagic diarrhoea following severe hypernatraemic dehydration				
	1½ weeks	2 weeks	8	
	4½ weeks	5 weeks	2	
Prolonged diarrhoea poor condition on referal Final Δ lactose intolerance				
1	4 weeks	7 weeks	8	
4	4½ weeks	6 weeks	1	
5	6 weeks	8 weeks	4	
6	6½ weeks	7½ weeks	7	
7	7 weeks	7½ weeks	6	Very undernourished Necrotic scalp lesion
8	14½ weeks	17½ weeks	3	
9	18 weeks	19½ weeks	4	
0	0 weeks	21 weeks	5	
Prolonged diarrhoea with transient monosaccharide intolerance				
11	2 days	2 weeks	79	
12	7 weeks	4 weeks	4	
13	2½ weeks	5½ weeks	4	
14	3 weeks	4 weeks	3	
15	4½ weeks	6 weeks	7	
16	5 weeks	6 weeks	7	
17	5 weeks	6 weeks	3	
18	7 weeks	8 weeks	7	
19	7 weeks	10 weeks	3	Increasing dehydration first 24 hours
20	7 weeks	12 weeks	6	Scalp abscess at drip site
1	7½ weeks	12 weeks	3	
22	9 weeks	12½ weeks	3	
23	9½ weeks	10½ weeks	7	Necrotic scalp lesion
24	10½ weeks	13½ weeks	4	
25	17½ weeks	13½ weeks	9	
6	12½ weeks	13½ weeks	10	Scalp infection at drip site
27	15 weeks	18½ weeks	3	
8	18 weeks	24 weeks	3	
29	23 weeks	24 weeks	15	In reasing dehydration & acidosis first 24 hours Scalp abscess at drip site
4 *Intractable diarrhoea*				
30	7 days	3 weeks	13	
31	7 weeks	4 weeks	0	Pulmonary oedema third day
	3 weeks	5½ weeks	76	Weight loss first 24 hours CVL after 8 days IVF
	3 weeks	11½ weeks	79*	Slough drip site hand Scalp abscess Septic arthritis (2 septic cutdown wound on referral)
	4 week	10 weeks	10	Slough drip site wrist
	5½ week	7 weeks	30	
	7 weeks	9½ weeks	42	CVL after 26 days IVF→septicaemia
	14 weeks	18 weeks	19	Inflammation leg after saphenous cutdown
	17 weeks	18½ weeks	21	
	21 weeks	25½ weeks	104	CVL after 2½ months intermittent IVF Slough ankle
	23 weeks	24 weeks	25	CVL af er 16 days IVF Died of septicaemia
	9 weeks	30½ weeks	67	CVL after 4 days IVF Died of septicaemia
	30 weeks	32½ weeks	8	Subsequent loose stools and failure to thrive on various diets Did well on soy preparations

Received IVF before referral Age at start and total days IVF refers to that in the unit
IVF=intravenous feeding CVL=central venous line

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INTRAVENOUS FEEDING OF YOUNG INFANTS WITH PERSISTENT DIARRHOEA

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ABSTRACT Banister A, Matin-Siddiqi, S A, Hatcher, G W and Hendrickse R G (*Department of Child Health*, Alder Hey Children s Hospital, Liverpool England) Intraven ous feeding of young infants with persistent diarrhoea Acta Paediatr Scand 64 732 1975 —42 infants with persistent diarrhoea were fed intravenously using a simplified regime based on Intralipid and an aminoacid, Fructose and ethanol solution Peripheral veins were used for up to 56 days, and with scalp veins complications were few and minor The use of arm and leg veins caused more frequent local problems and is not advised Central venous lines became necessary in 5 infants and 3 developed septicaemia The regime was well tolerated with adequate weight gain when intake was adjusted to the infants' needs Rates of infusion of 1 g Intralipid/kg hourly over 2 hours and up to 1 g fructose/kg hourly over 14 hours did not cause persistent lipaemia (except transiently in 2 infants) nor metabolic acidosis Infants must be fully rehydrated with correction of acidosis and electrolyte imbalance before starting intravenous feeding, or acidosis and dehydration from osmotic diuresis may occur Intraven ous feeding should be started gradually and cautiously in severely malnourished infants and should not be used where liver function is abnormal

KEY WORDS Infants protracted diarrhoea parenteral nutrition

Intravenous nutrition of children who cannot meet their needs by oral intake is an important therapeutic advance (7, 22) Where a central venous line is necessary for intravenous feed ing the high risk of septicaemia may lead to delay in the introduction of adequate nutrition in infants with malabsorptive problems

With the introduction of safe intravenous fat preparations which are non irritant and pro vide a high calorie intake in small volume it has been possible to use peripheral veins for long periods to provide adequate intravenous nutrition Harries (10) reviewed the problem and presented a regime using peripheral veins but requiring a very high level of nursing and medical supervision Wei et al (21) have had good results with a simpler regime We present our experience during the first two years since its introduction of a similar regime which has been found to be satisfactory in young infants

PATIENTS

42 infants admitted to the gastroenteritis unit in the two year period November 1969 to October 1971 received intravenous feeding for a total of 556 days Details are shown in Table 1 All 42 presented with a diarrhoeal illness These infants when compared with those whose diarrhoea settled without problems were all very young, the majority under two months old The younger the in fant the less likely it was for the initial illness to be se vere or for a bacterial pathogen to be isolated from the stool Thus in infants requiring intravenous feeding of those less than 8 weeks old 30% required intravenous rehydration initially compared with 60% of those more than 16 weeks old while 9% had faecal pathogens com pared with 40% of those over 16 weeks

The main indications for intravenous feeding were 1) to provide adequate nutrition while reaching a diagnosis and rational oral feeding in infants with diarrhoea already wasted when first seen 2) to avoid undernourishment in

Table 3 *Contents of intravenous feeding regimes compared with oral feeds All expressed per kg body weight per day*

	This series		Wei et al 1972	Harries 1971	Borreson et al 1970	Oral	Oral
Protein source	Amigen	AFE	(Amigen)	(Vamin)	(Aminofusin)	(Breast milk)[a]	(Powdered milk)[b]
Fluid ml	150	150	145	145	140	150	150
Calories	100	107	1?0	102	100	100	100
Protein or Amino acid g	4 7	3	6 25	3 5	3	2 4	5
Fat g	2	2	2	4	4	5 6	5
CHO g	13 4	15 7	15 6	15 5	12	10	10
Na mEq	4 7	6	4 3	2 5	1 05	0 8	3 2
K mEq	2 9	3 7	5	?	2 75	1 7	5 7
Cl mEq	4 8	6 5	2 75	2 5	?	1 6	4
Ca mEq	0 45	0 74	0 63	2	1	? 8	6
Mg mEq	0 18	0 33	1 75	0 45	0 15	0 67	0 6
P mEq	3	3 6	3 75	2 5	7 7	4 9	7

[a] Figures based on average of those quoted by Nelson Textbook of Paediatrics and Documenta Geigy
[b] Variable figures based on commonly used full cream preparations

persistent diarrhoea who developed hypokalaemia An increased intake of potassium was needed A four week old infant (no 31) received 0 45% saline in place of 0 18% saline and developed peripheral and pulmonary oedema which responded to *frusemide* and a reduced sodium intake Plasma calcium and phosphate remained normal except terminally in one infant who died of septicaemia after 67 days of intravenous feeding Serum transaminase levels were slightly raised in two infants on prolonged intravenous feeding but were otherwise normal

In two infants only was it necessary temporarily to reduce the dose of Intralipid because of persisting lipaemia Losses of fructose and aminoacids in the urine were slight Measurement of fructose in the urine over a 24 hour period in six infants on full intravenous feeding with AFE for more than a week showed a loss of 0–2 5% of the intake The loss of aminoacids in 5 infants over 3 months of age was less than 1 2% of the intake One 4 week old infant lost 6 8% of the intake

Two infants (nos 40 and 41) died of infection associated with a central venous line The liver of infant no 40 showed scattered minor fatty changes

Vein tolerance

Most infants had received intravenous fluids before intravenous feeding commenced some for long periods before referral and therefore not all had suitable peripheral veins However peripheral veins were regularly used for intravenous feeding for 2–3 weeks even in young infants and one patient was fed by this route for 56 days The use of an infusion pump (IVAC 500 Tekmar Medical) prolonged the life of the individual drip

The average life of a scalp vein drip using the AFE regime was 25 hours (29 infusions) without an infusion pump and 37 6 hours (52 infusions) with a pump The difference is significant ($p<0\ 02$ using Student s t test) Using Amigen 800 with a pump the average life of a drip was 40 5 hours (31 infusions)

There were few complications with scalp vein drips Two infants developed small necrotic scalp lesions at the site of needle insertion and three others developed small superficial scalp abscesses All these healed well One infant developed infection and spreading phlebitis at the drip site and in this case the infusion pump may have been responsible for spreading an infection which might have remained localised

Experience of intravenous feeding through

Table 2 *Intravenous feeding regime*

AFE=Aminosol fructose ethanol

Substance	Dose	
Intralipid 20%	10 ml/kg	Daily (5 ml/kg hourly)
Amigen 800 or AFE	90 ml/kg	Daily (6 5 ml/kg hourly)
0 18% saline in 4 3% dextrose plus KCl 1 g in 500 ml	50 ml/kg	Daily (6 5 ml/kg hourly)
Pabrinex* 1&2	1 ml each	IV twice weekly in dextrose electrolyte
Vitamin B12	5 μg	IV twice weekly in dextrose electrolyte
Vitamin K	1 mg	IV twice weekly in dextrose electrolyte
Folic acid	10 mg	IM weekly
Imferon	0 5 ml	IM every 2 weeks
Plasma	20 ml/kg	IV every 2 weeks

Potassium chloride 1 g in 500 ml AFE added from the start After 1 week the following is added to each 500 ml AFE instead (1) Potassium phosphate 13 mEq of K (2) Magnesium sulphate 10% 2 ml and (3) Calcium gluconate 10% 10 ml

* Parenteral multivitamin preparation (Paines and Byrne)

those where adequate oral intake could not be achieved within a reasonable time At the start of intravenous feeding it was not possible to distinguish those infants with transient problems needing intravenous feeding for only a few days from those with prolonged intractable diarrhoea

INTRAVENOUS FEEDING REGIME

Intravenous feeding (see Table 2) was based on Intralipid[1] and either Aminosol Fructose Ethanol[1] (AFE) or Amigen 800 [2] This regime is compared with others suitable for use through peripheral veins in Table 3

Method

1 Blood samples were collected in the morning to check daily for lipaemia by inspection of plasma and for urea electrolyte and bicarbonate levels With prolonged intravenous feeding plasma calcium magnesium and phosphate and serum transaminases were measured periodically

2 The 24 hour sequence began mid-day with Intralipid given over 2 hours followed by the aminoacid solution given over the next 13–14 hours followed by the glucose electrolyte solution given over 8–9 hours The glucose electrolyte solution could be altered in content and/or volume as required

3 Scalp veins were used preferentially and for as long as possible

4 All drip tubing and solutions were changed daily The Intralipid was given through a separate drip set

5 Intravenous vitamins were conveniently added to the glucose electrolyte solution in the burette of the giving set.

6 Where intravenous antibiotics and other drugs were necessary they were given through a three way tap kept closed between injections by a sterile syringe

7 Dehydration acidosis and electrolyte imbalance were corrected and hypoproteinaemia treated with intravenous plasma or albumin before starting intravenous feeding Blood glucose and urine reducing substances were checked during the introduction of feeding

8 In malnourished infants intravenous feeding was gradually increased to that for expected weight

Early in the series intravenous feeding was started after ten days of inadequate nutrition a half dose of Intralipid was used on the first day and 70 ml/kg of aminoacid preparation was given daily With increasing confidence in the regime intravenous feeding was started on the fifth day if oral intake was still not established and the full regime given from the start For part of the series there were difficulties in obtaining supplies of Amigen These have been overcome and Amigen is the preparation now in regular use in the unit

RESULTS

The regime was generally well tolerated T average daily weight increase of patients complete intravenous feeding for six days more is shown in Table 4 Weight gain w poor in those receiving less than 80 ml/ aminoacid solution per day and weight tend to be static during episodes of infectio Weight gain was also poor during periods diarrhoea provoked by repeated unsuccessf attempts to establish oral feeding unless i take was increased Infants appeared to r spond similarly to intravenous feeding bas on either Amigen 800 or AFE

No infant who was rehydrated before start of intravenous feeding showed metab acidosis and hypoglycaemia was not seen three infants (nos 19 29 and 32) intraven feeding was started before correction of hydration One lost further weight but was otherwise ill The other two became more hydrated and developed tachypnoea metabolic acidosis It seems that the fruct aminoacid mixture in the presence of deh ration produced an osmotic diuresis acidosis

Electrolyte levels remained normal dur intravenous feeding except in two infants w

[1] Paines and Byrne

[2] Baxter

roduction of feeds

npts to reintroduce oral fluids were usu)nly made when diarrhoea had ceased for ast 24 hours One or two feeds of boiled r were given and if tolerated weak glu or fructose solutions were tried If a 5% osacchande solution was tolerated then appropriate low lactose feed was usually n in dilute form gradually increasing to strength Nine infants tolerated 5%)se and a standard cow s mild preparation introduced

was convenient to introduce oral fluid in nl amounts three hourly reducing the in enous glucose electrolyte allowance pro tionately and giving the aminoacid solu i more slowly reducing the drip rate by 10 hour As soon as half or full strength feeds re tolerated in this small volume intraven feeds were discontinued and the oral vol e built up

Where monosacchandes could not be intro ced after a few attempts an oral aminoacid paration Albumaid Complete (Scientific spital Supplies) was tried then medium ain triglycerides added followed later by cautious introducton of a monosaccha le The infants were kept on this artificial xture for a time before conversion to a w s milk or low lactose preparation Three tients (nos 33 39 and 42) were intolerant cow s milk protein and successfully fed ith soy preparations Where jejunal biopsy owed a flat mucosa gluten was excluded om the diet for some months

Each new substance was given for at least 4 hours before making a change as intoler nce did not always show immediately

DISCUSSION

n planning this regime of intravenous feeding he aim was to achieve satisfactory nutrition sing the smallest number of solutions and to eep additions to a minimum to avoid the risk f infection and administrative errors Amigen 800 and AFE have a similar aminoacid fructose and alcohol content but whereas the former contains adequate potassium and reasonable amounts of calcium magnesium and phosphorus the latter contains little potassium and virtually no calcium or magnesium Amigen 800 is therefore prefer red to AFE Although Amigen has the great advantage of a reasonable mineral content balance data in 4 infants studied by Borreson et al (4) suggest that an intake of 1 5–2 mEq calcium and 0 3 mEq magnesium per kg per day are needed for positive balance Infants receiving Amigen for more than a week should probably therefore have added calcium and magnesium salts and more calcium should be given in the AFE regime

We feel that the dextrose saline fraction of the regime allows flexibility in correcting fluid or electrolyte losses and also makes the intro duction of oral intake a simple matter while still fully nourishing the infant

Wei et al (21) have used a similar regime with Intralipid and Amigen 800 alone using 125 ml per kg per day of Amigen over 20 hours and report satisfactory results in 23 children Their metabolic studies have shown that the various nutrients are adequately metabolised

Intralipid was used as a concentrated source of calories and its use presented few problems Attempts to run Intralipid and aminoacid pre parations together through a Y tube proved unsatisfactory and intermittent alternate administration of solutions was without spe cial advantage and unnecessarily difficult for the nursing staff The 24 hour requirement of Intralipid was given alone over a two hour period after obtaining the results of the day s blood samples followed by the aminoacid sol ution and the glucose electrolyte allowance This cycle of feeding ensured a high calorie supply for maximum utilisation of infused aminoacids and plasma samples were clear of lipaemia by the following morning when laboratory investigations were done A further advantage was that changing of drip sets giv ing of Intralipid vitamins and any modifica

Table 4 *Weight gain in 22 infants during periods of intravenous feeding lasting 6 days or m*

No	Age (weeks)	Days IVF	Regime (ml/kg)		Average wt gain (g/day)	Comments
18	8	7	AFE	70	0	
31	4	15	AFE	70	0	Bronchiolitis
40	24	25	AFE	75	0	Septicaemia
36	9½	42	AFE	70	6 2	
16	6	7	Amigen	90	7 1	
34	10	10	AFE	70	7 7	Repeated oral challenge with diarrhoea
41	30½	67	AFE	75	8 3	Septicaemia
39	25½	16	Amigen	90	11 9	
		9	Amigen	115	29	Repeated oral challenge with diarrhoea
		10	Amigen	135	32	Intralipid also increased to 14 ml/kg/day
35	7	12	Amigen	90	15 5	Intralipid also increased to 17 ml/kg/day
		14	Amigen	90	53	
26	13½	10	AFE	90	17	
37	18	9	AFE	90	17 7	Scalp sepsis
30	3	13	AFF	80	26 3	
38	18½	21	AFE	90	26 7	
32	5½	21	AFF	80	27 2	
15	6	7	AFE	80	28 6	
20	12	6	AFE	90	31	
25	13½	9	AFE	90	31 2	
23	10½	7	Amigen	90	32 6	
29	24	9	AFE	90	34 6	
		6	AFE	90	51 8	
1	2	8	AFE	90	35 3	
33	11½	16	Amigen	90	39	
		8	AFE	90	27 5	
3	7	8	Amigen	90	40	

IVF=Intravenous feeding

veins on the hands and feet in these young infants was unsatisfactory These sites were rarely used, but on three occasions necrotic lesions occurred following leakage of amino acid solutions, and one required skin grafting The life of a saphenous cutdown infusion was less than 48 hours on the 4 occasions it was used In one infant the lower leg became swollen red indurated and tender for several days Infection was unlikely to have caused this reaction as blood cultures were negative and the inflammation settled without antibiotics

Central venous infusions

Central venous infusions were used in five infants when peripheral veins were no longer available Despite strict aseptic technique in handling these drips three of the five infants developed septicaemia One was infected with streptococcus viridans after the line had been in 18 days He did well with removal of the catheter and antibiotics Another developed a haematoma around the venous insertion of the catheter which became infected She died of septicaemia and staphylococcal lung absce ses despite removal of the line and appropria antibiotics

The third infant was suffering from stapl ylococcal enteritis and pneumonia on referra Gross oedema made the use of peripher veins impossible and a central venous lin was inserted despite the presence of systemi infection During the 67 days of intravenous feeding when she was intolerant of all ora intake she also developed candida septi caemia and pseudomonas infection which was eventually fatal The first line was re moved after 26 days and peripheral veins used for another 27 days before a second central venous line had to be inserted

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tion of the aminoacid solution were all performed by senior day staff, and night staff usually only had to change over to the glucose electrolyte solution Wei et al (21) gave the same dose of Intralipid with heparin over 4 hours They found blood triglyceride and free fatty acid levels fell to normal 2–4 hours after stopping Intralipid infusion We do not know how rapidly the fat was cleared in our infants There were no apparent ill effects from giving the solution at a rate of 1 g/kg hourly

The commercial solutions used have fructose as their source of carbohydrate calories The final steps of metabolism are the same for fructose and glucose but some workers feel that fructose has particular dangers in children Certainly high doses of fructose may produce dehydration from osmotic diuresis as may glucose (1) and cause metabolic acidosis (19) Reports of trouble in children have been where rapid infusions of fructose have been given to dehydrated patients We have only had trouble with our regime where it has been started before dehydration and existing metabolic acidosis have been corrected in all other cases a rate of nearly 1 g fructose/kg hourly has been well tolerated and there has been no metabolic acidosis in blood samples within five hours of completing the fructose infusion Wei et al (21) found fructose was well utilised by their patients Plasma pyruvic and lactic acid levels were slightly raised at up to twice normal no metabolic acidosis occurred We consider that problems with fructose are only likely to be seen in dehydrated or severely malnourished infants who may have impaired liver function where osmotic diuresis and lactic acidosis may occur

The alcohol content of Amigen 800 and AFE provides a further useful source of calories Wei et al (21) showed that all their patients were able to metabolise alcohol as rapidly as it was given and blood levels fell to zero within 50 minutes of stopping the infusion

The ideal aminoacid solution for intravenous feeding at any particular age has not yet been devised The long term effects on the *growing brain of the altered plasma* aminoacid levels seen in intravenous feeding are not yet known Hydrolysed casein as in Amigen and fibrin as in Aminosol provide adequate supplies of essential aminoacids for the growing infant (12) in the usual amounts given and are widely used (21 18) Although the newer synthetic aminoacid mixtures hold out hopes of devising an ideal solution for infants in the *future* their use so far has not been without problems Metabolic acidosis may occur due to the excess of cationic aminoacids in the solution (11) These solutions at present are of relatively low calorie content and require the addition of electrolytes and minerals before use

Sepsis was a serious problem in our patients who had a central venous line and other authors have reported similar serious problems (2 8 9 10) Although Nelson (18) has reduced the incidence of septicaemia to 9% by scrupulous technique this risk is still high However intravenous feeding can be safely given for long periods through peripheral veins even in young infants Our experience using scalp veins has been similar to that of Wei et al (20) who had no sepsis and only two instances of local tissue necrosis *in 25 children* when using peripheral veins We feel that veins in the arms and legs are best avoided in infants because of difficulties in immobilisation and the risk of needle dislodgement and leakage of irritant solutions into surrounding tissues

With a relatively safe method there are good theoretical reasons for establishing adequate *intravenous nutrition early* when oral intake is inadequate Malabsorption in infants following gastroenteritis may lead to rapid deterioration in nutritional state with hypoproteinaemia and increased susceptibility to infection and malnutrition may in turn increase malabsorption Intestinal damage directly attributable to malnutrition in childhood includes villous atrophy (3 6) low jejunal disaccharidase

activity and clinical lactose intolerance (5 14) poor glucose absorption (15) and reduced je junal dipeptide hydrolysis (17) Even in obese adults 2–4 weeks of therapeutic starvation causes reduction in jejunal disacchandases (16) Apart from the possible ill effects of malnutrition on the gastrointestinal tract seri ous nutritional disturbance at a time of rapid brain growth and myelination of the central nervous system may have permanent effects (23)

Infants with simple secondary lactose intol erance should not become ill and wasted as the result of delay in diagnosis and alteration in diet However in more complex problems of malabsorption the introduction of intraven ous feeding is sometimes delayed while re peated attempts are made to introduce oral feeds which are poorly tolerated Our experi ence suggests that apart from increasing malnutrition and episodes of dehydration and electrolyte depletion undue persistence with unsuccessful oral feeding may increase the malabsorptive problem Eleven patients who initially tolerated 150 ml per kg of 4 3% glu cose per day by mouth became glucose intol erant when milk feeds were introduced and con ued in spite of diarrhoea In patients on intravenous feeding persistent unsuccessful oral challenge also appeared to make matters worse A period of full intravenous nutrition and complete gut rest greatly improved the infants general condition and may have shortened the period of monosacchande and other intolerances in some cases

The Amigen regime has now been satis factorily used in the unit for a further 3 years Some infants have received up to 4 g Intralipid and 120 ml Amigen per kg daily A drop in platelets has been seen in some cases this has had possible clinical significance in one infant During the 3 years only one infant fed intravenously died a baby with immune deficiency and pneumocystis carinii infection This intravenous regime is simple and within the competence of any unit experienced in in travenous therapy of infants Apart from its life saving use in established severe malabsorptive problems (13) we feel that its early use should result in infants being maintained in good condition throughout po tentially serious illness and may well shorten the malabsorptive state Fuller investigation should be possible in well nourished infants with malabsorptive problems than is usually justified at present and the reintroduction of oral substances should become a planned and informative process With the gradual intro duction of oral feeds while maintaining nutri tion hypoglycaemia on stopping intravenous feeds (21 10) should not be seen

This regime should only be started after full correction of dehydration acidosis and elec trolyte imbalance should be introduced grad ually and cautiously in severely malnourished infants and should not be used where liver function is abnormal

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REFERENCES

1 Andersson G Brohult J & Sterner G Increasing metabolic acidosis following fructose infusion in two children *Acta Paed atr Scand 58* 301 1969
2 Ashcroft K W & Leape L L Candida sepsis omplicating parenteral feeding *JAMA* 212 454 1970
3 Berkel I Kiran O & Say B Jejunal mucosa in infant le malnutrition *Acta Paed atr Scand* 59 58 1970
4 Borresen H C Coran A G & Knutrud O Metabolic results of parenteral feeding in neonatal surgery *Ann Su g 172* 91 1970
5 Bowie M D Brinkman G L & Hansen J J Acqu red disaccharide intolerance in malnutrition *J Ped atr 66* 1083 1965
6 Brunser O Re d A Monkberg F Maccioni A & Contreras I Jejunal biopsies in infant malnutrition w th special reference to mitot c index *Ped at cs* 38 605 1966
7 Filler R M Eraklis A J Rubin V G & Das J B Parenteral nutrition of infants *N Engl J Med* 281 589 1969

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With a relatively safe method there are good theoretical reasons for establishing adequate intravenous nutrition early when oral intake is inadequate Malabsorption in infants following gastroenteritis may lead to rapid deterioration in nutritional state with hypoproteinaemia and increased susceptibility to infection and malnutrition may in turn increase malabsorption Intestinal damage directly attributable to malnutrition in childhood includes villous atrophy (3 6) low jejunal disaccharidase

ity and clinical lactose intolerance (5 14) r glucose absorption (15) and reduced je l dipeptide hydrolysis (17) Even in obese lts 2-4 weeks of therapeutic starvation ses reduction in jejunal disaccharidases) Apart from the possible ill effects of lnutrition on the gastrointestinal tract seri nutritional disturbance at a time of rapid in growth and myelination of the central vous system may have permanent effects)

nfants with simple secondary lactose intol ance should not become ill and wasted as result of delay in diagnosis and alteration diet However in more complex problems malabsorption the introduction of intraven s feeding is sometimes delayed while re ated attempts are made to introduce oral eds which are poorly tolerated Our experi ice suggests that apart from increasing alnutrition and episodes of dehydration and ectrolyte depletion undue persistence with nsuccessful oral feeding may increase the ialabsorptive problem Eleven patients who nitially tolerated 150 ml per kg of 4 3% glu ose per day by mouth became glucose intol rant when milk feeds were introduced and ontinued in spite of diarrhoea In patients on ntravenous feeding persistent unsuccessful ral challenge also appeared to make matters vorse A period of full intravenous nutrition nd complete gut rest greatly improved the nfants general condition and may have shortened the period of monosaccharide and other intolerances in some cases

The Amigen regime has now been satis factorily used in the unit for a further 3 years Some infants have received up to 4 g Intralipid and 120 ml Amigen per kg daily A drop in platelets has been seen in some cases this has had possible clinical significance in one infant During the 3 years only one infant fed intravenously died a baby with immune deficiency and pneumocystis carinii infection This intravenous regime is simple and within the competence of any unit experienced in in travenous therapy of infants Apart from its life saving use in established severe malabsorptive problems (13) we feel that its early use should result in infants being maintained in good condition throughout potentially serious illness and may well shorten the malabsorptive state Fuller investigation should be possible in well nourished infants with malabsorptive problems than is usually justified at present and the reintroduction of oral substances should become a planned and informative process With the gradual introduction of oral feeds while maintaining nutri tion hypoglycaemia on stopping intravenous feeds (21 10) should not be seen

This regime should only be started after full correction of dehydration acidosis and elec trolyte imbalance should be introduced grad ually and cautiously in severely malnourished infants and should not be used where liver function is abnormal

ACKNOWLEDGEMENTS

We are grateful to Mr Goodman Chief Pharmacist and Miss Clothier Dietician for invaluable help and to Mr Ireland Biochemist for measurement of urinary aminoacids We particularly wish to thank Sister Garawal and the other nursing staff for their skilled and devoted care of these babies

REFERENCES

1 Andersson G Brohult J & Sterner G Increasing metabolic acidosis following fructose infusion in two children *Acta Paediatr Scand 58* 301 1969
2 Ashcroft K W & Leape L L Candida sepsis complicating parenteral feeding *JAMA 212* 454 1970
3 Berkel I Kiran O & Say B Jejunal mucosa in infantile malnutrition *Acta Paediat Scand 59* 58 1970
4 Borresen H C Coran A G & Knutrud O Metabolic results of parenteral feeding in neonatal surgery *Ann Surg 172* 291 1970
5 Bowie M D Brinkman G L & Hansen J L Acquired disaccharide intolerance in malnutrition *J Pediatr 66* 1083 1965
6 Brunser O Reid A Monkberg F Maccioni A & Contreras I Jejunal biopsies in infant malnutrition with special reference to mitotic index *Pediatrics 38* 605 1966
7 Filler R M Eraklis A J Rubin V G & Das J B Parenteral nutrition of infants *N Engl J Med 281* 589 1969

tion of the aminoacid solution were all performed by senior day staff and night staff usually only had to change over to the glucose electrolyte solution Wei et al (21) gave the same dose of Intralipid with heparin over 4 hours They found blood triglyceride and free fatty acid levels fell to normal 2–4 hours after stopping Intralipid infusion We do not know how rapidly the fat was cleared in our infants There were no apparent ill effects from giving the solution at a rate of 1 g/kg hourly

The commercial solutions used have fructose as their source of carbohydrate calories The final steps of metabolism are the same for fructose and glucose but some workers feel that fructose has particular dangers in children Certainly high doses of fructose may produce dehydration from osmotic diuresis as may glucose (1) and cause metabolic acidosis (19) Reports of trouble in children have been where rapid infusions of fructose have been given to dehydrated patients We have only had trouble with our regime where it has been started before dehydration and existing metabolic acidosis have been corrected in all other cases a rate of nearly 1 g fructose/kg hourly has been well tolerated and there has been no metabolic acidosis in blood samples within five hours of completing the fructose infusion Wei et al (21) found fructose was well utilised by their patients Plasma pyruvic and lactic acid levels were slightly raised at up to twice normal no metabolic acidosis occurred We consider that problems with fructose are only likely to be seen in dehydrated or severely malnourished infants who may have impaired liver function where osmotic diuresis and lactic acidosis may occur

The alcohol content of Amigen 800 and AFE provides a further useful source of calories Wei et al (21) showed that all their patients were able to metabolise alcohol as rapidly as it was given and blood levels fell to zero within 50 minutes of stopping the infusion

The ideal aminoacid solution for intravenous feeding at any particular age has not ye been devised The long term effects on th growing brain of the altered plasma aminoaci levels seen in intravenous feeding are not ye known Hydrolysed casein as in Amigen anc fibrin as in Aminosol provide adequate supplies of essential aminoacids for the growing infant (12) in the usual amounts given and are widely used (21 18) Although the newer synthetic aminoacid mixtures hold out hopes of devising an ideal solution for infants in the future their use so far has not been without problems Metabolic acidosis may occur due to the excess of cationic aminoacids in the solution (11) These solutions at present are of relatively low calorie content and require the addition of electrolytes and minerals before use

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MEASUREMENT OF SYSTOLIC BLOOD PRESSURE IN FINGERS OF NEWBORN INFANTS

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ABSTRACT Gundersen J and Dahlin K (Departments of Surgery Paediatrics and Clinical Physiology General Hospital S-21401 Malmo, Sweden) Measurement of systolic blood pressure in fingers of newborn infants Acta Paediatr Scand 64 741 1975 —The systolic blood pressure in the index finger was measured using a minicuff and a strain gauge both placed around the finger 122 measurements in four newborn infants were performed during monitoring in an incubator and the systolic blood pressure values obtained were compared with the simultaneously recorded intra arterial blood pressure in the aorta. Good correlation between direct and indirect pressure values was found An important advantage is that the measurements do not arouse the infants nor disturb their sleep The procedure seems suited for further technical development and automatic measurements

KEY WORDS Blood pressure finger blood pressure perinatal care monitoring

Because of the low blood pressure during the first year of life palpation of the pulse is difficult and no Korotkoff sounds are audible when measuring the indirect blood pressure using an arm cuff and a stethoscope (10) Direct measurements of blood pressure can seldom be used the danger of thrombo embolism caused by indwelling catheters (3 9) is a serious drawback

Many attempts have been made to record the indirect blood pressure in infants The flush method (11) has been widely used but is a coarse and cumbersome procedure Celander & Thunell (1 2) improved the pulse detection by using a mercury in rubber strain gauge placed around the calf and thus measured the systolic blood pressure in the thigh with a cuff We found that this technique often made the infants cry and move the limbs which makes the pulse detection difficult We therefore tried to measure the blood pressure in the arm using the same technique but experienced similar problems of crying and agitation during the measurement With simultaneous recording of the intra arterial blood pressure it was evident that the indirect measurement of the arm blood pressure caused a considerable rise of the systolic and diastolic pressure values

As measurement of the systolic blood pressure in fingers and toes has proved to be sufficiently sensitive to record even the low pressures in the digits of limbs with advanced arterial insufficiency (5 6 7) and to cause the patient no discomfort it was decided to construct special cuffs for the fingers of newborn infants It was the aim of this investigation to analyse the reliability of blood pressure measurements in the index finger by comparing the values with those obtained from simultaneously recorded intra arterial blood pressure

MATERIAL AND METHOD

In some newborn infants an intra arterial catheter was introduced into the aorta through the umbilical artery in

8 Filler R M & Eraklis A J Care of the critically ill child intravenous alimentation *Pediatrics 46* 456 1970
9 Groff D B Complications of intravenous hyper alimentation in newborn infants *J Pediatr Surg 4* 460 1969
10 Harries J T Personal practise Intravenous feeding infants *Arch Dis Childh 46* 855 1971
11 Heird W C Dell R B Driscoll J M Grebin B & Winters R W Metabolic acidosis resulting from intravenous alimentation mixtures containing synthe tic aminoacids *N Engl J Med 287* 943 1972
12 Heird W C Driscoll M D Schullinger J N Grebin B & Winters R W Intravenous alimenta tion in pediatric patients *J Pediatr 80* 351 1972
13 Hyman C J Reiter J Rodnan J & Drash A L Parenteral and oral alimentation in the treatment of the nonspecific protracted diarrheal syndrome of in fancy *J Pediatr 78* 17 1971
14 James W P T Jejunal disaccharidase activities in children with marasmus and with kwashiorkor Re sponse to treatment *Arch Dis Childh 46* 218 1971
15 James W P T Comparison of three methods used in assessment of carbohydrate absorption in malnourished children *Arch Dis Childh 47* 531 1972
16 Knudsen K B Bellamy H M Lecocq F R & Bradley E M The influence of fasting and refeeding on jejunal disaccharidases *Clin Res 14* 300 1966
17 Kumar V Ghai O P & Chase H P Intestinal dipeptide hydrolase activities in undernourished children *Arch Dis Childh 46* 801 1971
18 Nelson R Minimising systemic infection durin complete parenteral alimentation of small infants *Arch Dis Childh 49* 16 1974
19 Sahebjami H & Scalettar R Effects of fructose infusion on lactate and uric acid metabolism *Lancet I* 366 1971
20 Wei P Hamilton J R & Le Blanc A E Intraven ous feeding of infants and children by periphera vein *Annual meeting of the Royal College of Physi cians and Surgeons of Canada* 1971
21 Wei P Hamilton J R & Le Blanc A E A clinical and metabolic study of an intravenous feeding techni que using peripheral veins as the initial infusion site *Can Med Assoc J 106* 969 1972
22 Wilmore D W Groff D B Bishop H C & Dudrick S J Total parenteral nutrition in infants with catastrophic gastrointestinal anomalies *J Pediatr Surg 4* 181 1969
23 Winick M Rosso P & Waterlow J Cellula growth of cerebrum cerebellum and brain stem i normal and marasmic children *Exp Neurol 26* 393 1970

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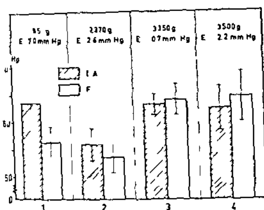


Fig 3 Mean values of systolic blood pressure recorded intraarterially (I A) and in the index finger (F) E=the difference between the mean values of intra arterial and finger pres ure The weights for the four infants are indi ated above Vertical bars=S D

the intra arterial blood pressure Sometimes the infants were awake and moved the arm which caused noise from the gauge As such spontaneous periods of activity are often inter rupted by periods of rest a few minutes ex pectancy solved this problem It was only oc casionally necessary to stabilize the arm while measuring

When the same cuff is used for fingers of fferent diameter it should be expected that ie smaller the diameter of the finger the igher the recorded blood pressure (4 5) This as also found to be true in the present in estigation In cases 1 and 2 the directly re orded pressures were higher than the indirect alues In cases 3 and 4 the directly recorded alues were slightly lower than the indirect ines The spread of both direct and indirect alues is higher in case 4 than in the other ases The explanation for this is some fluctu tions of the intra arterial blood pressure hich demonstrates that this infant was not in completely steady state

When discussing the reliability of indirect measurements of blood pressure two problems must be considered the reproducibility and the error In this series the reproducibility seems to be good as expressed by the low S D of the indirect pressure values The difference between mean values of direct and indirect blood pressure (E) was from 7 to −2 mmHg and it was found that with increasing weight the difference (E) decreased (Fig 3) As it may be assumed that in infants the finger diameter increases with the body weight it seems possi ble to correct the finger blood pressure to true pressure values when the weight is known It should however be borne in mind that there are several errors involved with di rect recording of blood pressure This implies that the intra arterial blood pressure may not always be the true blood pressure which may also modify the conception of error in this connection

Measuring the blood pressure in the index finger of infants therefore seems to be both simple and reliable The error is of the same magnitude as when the blood pressure is measured in the arm with the aid of a standard cuff in subjects with different diameters of the arm and the pressure is compared with the intra arterial values The technique seems suited for monitoring newborn infants as measurements may readily be performed in an incubator The procedure may be further de veloped for automatic measurements

REFERENCES

1 Celander O & Thunell G A plethysmographic method for measuring the systolic and diastolic blood pressure in newborn infants A ta Paedictr Scand 49 497 1960
2 — The mercury-in rubber strain gauge for measure ments of blood pressure and peripheral circulation in newborn infants Acta Paediatr Scand 50 505 1961
3 Garfield H A Stern R & Friis Hansen B Frekvens af tromboser og signifikante infektioner hos nyfødte behandlede med navlevene og arteriekateter Ugeskr Laeg Meddelelser fra Dansk Medicinsk Sel kab nr 5 59 1973
4 Gaskell P & Krisman A M The brachial to digital blood pressure gradient in normal subjects and in patients with high blood pressure Can J Biochem Phys ol 36 869 1958
5 Gundersen J Segmental measurements of sy tolic blood pres ure in the extremities including the thumb and the great toe Thesis Acta Chir Scand Suppl 4 6 197
6 — Mea urement of blood pressure in all the toes VASA 1 47 197
7 — Measurement of blood pressure in all fingers Dan sh M d Bull 20 1 9 1973

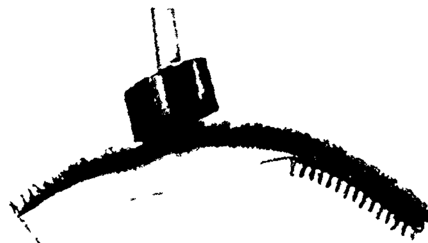


Fig 1 Minicuff for measuring finger blood pressure in newborn infants

order to obtain repeated blood samples for gas analyses The indications for this were small size for dates premalurity or respiratory complications Direct blood pressure recordings were made using an EMT transducer and a Mingograf 800 (Siemens Elema)

Simultaneously the indirect blood pressure was measured in the index finger using the following technique A 10 mm wide occlusion cuff was placed around the proximal phalanx (Fig 1) For details concerning the cuff see Gundersen (5) The cuffs were made of different lengths and cuffs with bladders completely encircling the digit were always used

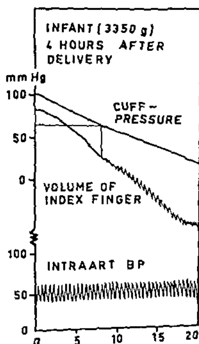


Fig 2 Recording of simultaneous intra arterial blood pressure and indirect blood pressure Comparison between direct and indirect pressure values is made from the same heartbeat i e the one which first can be detected during indirect measurement

Pulse detection was made by a mercury in silastic strain gauge plethysmograph placed with slight tension on the same finger distally to the cuff The gauge was connected to an electronic unit (8) placed in the same device used for direct pressure recording This technique allowed comparison of simultaneously recorded direct and indirect pressure values (Fig 2)

Four infants were studied Their sexes weights and number of measurements performed (n) were as follows No 1 female 1 850 g n=60 No 2 female 2 320 g n=70 No 3 male 3 350 g n=17 No 4 male 3 500 g n=25 The infants were placed in incubators and measurements performed under resting conditions which means at least 10 minutes after disturbing the infant Measurements were performed every 3 minutes

RESULTS

The mean systolic pressure values in infants studied are shown in Fig 3 The infants could be studied during sleep and it was therefore assumed that they were in steady states It appears that in each infant the direct and indirect pressure values are quite constant with a small variation as expressed by the S D Furthermore the difference between the mean values of direct and indirect blood pressure (E) decreases with increasing body weight

DISCUSSION

The study demonstrated that measurements of the systolic blood pressure in the index finger may be performed without technical problems and without disturbing the sleep or influencing

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NUTRITION IN LOW BIRTH WEIGHT INFANTS

III *Lipolysis and Free Fatty Acid Elimination after intravenous Administration of Fat Emulsion*

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ABSTRACT *Olegård R Gustafson A Kjellmer I* and *Victorin L* (Department of Paediatrics I University of Goteborg Goteborg Sweden) Nutrition in low birth-weight infants III Lipolysis and free fatty acid elimination after intravenous administration of fat emulsion Acta Paediatr Scand 64 745 1975.—Triglyceride linoleic acid in a fat emulsion for intravenous administration (Intralipid®) was used as a marker in an evaluation of fat metabolism in newborn low birth weight (LBW) infants Qualitative data on fatty acids as well as quantification of triglycerides and free fatty acids were obtained by gas-liquid chromatography Influences on these parameters after a single and after repeated injections of Intralipid® revealed differences between low birth weight infants appropriate for-date (AFD) ($n=8$) and those light for-date (LFD) ($n=5$) The LFD exhibited in comparison with the AFD infants an impaired lipolysis of injected triglycerides and a retarded elimination from plasma of released free fatty acids In LFD in general this resulted in triglyceride accumulation and low free fatty acid levels. Heparin facilitated plasma triglyceride lipolysis and free fatty acid elimination from the blood stream

KEY WORDS Neonatal metabolism nutrition low birth weight infant light for-date fat emulsion triglycerides free fatty acids heparin

The ability of low birth weight (LBW) infants to eliminate fat supplied parenterally as Intralipid® has been evaluated in earlier studies (3 4) In pre term infants with a birth weight that was appropriate for-date (AFD) the calculated maximal capacity of fat removal after a single injection was 0 3 g fat/kg b w /h Light for-date infants (LFD) revealed after a single injection a reduced capacity for fat elimination In AFD infants repeated injections for 6–8 hours of half the amount corresponding to the theoretical maximal capacity (0 15 g fat/kg b w /h) was well tolerated (4) while the same amount caused an accumulation of lipids in plasma in LFD infants unless heparin was administered In these earlier studies only to l lipids and turbidimetry were measured

In plasma all lipids are transported in the form of plasma lipoproteins Triglycerides (TG) are transported in chylomicrons and very low density lipoproteins (VLDL) TG in chylomicrons and VLDL are metabolized by hydrolysis through the action of lipo protein lipase into glycerol and free fatty acids (FFA) Intralipid particles agree in shape and size with chylomicrons and are metabolized in a similar way (5) FFA are transported in complex with albumin During fasting the major portion of FFA is derived from adipose tissue Released FFA are taken up by the liver adipose tissue and skeletal muscle In the new born infant the content of the essential fatty acid linoleic acid 18 2(n 6) is low in plasma TG FFA and adipose tissue (6) Furthermore no increase of FFA linoleic acid occurs in plasma from endogenous release (6) Thus the high linoleic acid content in Intralipid® can be used as a marker in the metabolism of Intralipid® (Table 1)

The present study was designed to evaluate

8 Gundersen J & Kendrup I Sträckpletysmografen—ett allsidigt medicinskt instrument *Läkartidningen 70* 2297 1973
9 Jacobsson B *Thromboembolism in vascular catheterisation* Thesis Göteborg 1969
10 Moss A J & Adams F H *Problems of blood pressure in childhood* C C Thomas Springfield Ill 1962
11 Weaver J C & Bohr D F The digital blood pressure I Values in normal subjects *Am Heart J 39* 431 1950

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ble 2 *Single injection Various plasma lipid data before and after a single injection of* *tralipid® (0 10 g fat/kg b w /h) in 8 AFD and 5 LFD infants*

	AFD ($n=8$)		LFD ($n=5$)		Significance of difference
	Mean	S E M	Mean	S E M	
einjectional TG mg/100 ml	36	8 6	57	7 3	N S
ostinjectional peak TG mg/100 ml	197	60 7	163	30 0	N S
einjectional FFA mg/100 ml	18	6 4	?0	4 9	N S
ostinjectional peak FFA mg/100 ml	6	7 1	30	5 0	N S
einjectional 18 2% in TG	5 1	0 8	10 7	1 5	$p<0$ 005
ostinjectional max 18 2% in TG	38 1	3 5	33 0	3 8	N S
einjectional 18 2% in FFA	3 7	0 4	5 9	0 6	$p<0$ 005
ostinjectional max 18 2% in FFA	19 9	?6	13 8	?0	N S
ostinjectional max 18 2% in FFA within 10 min	14 8	3 2	4 0	0 9	$p<0$ 05
G linoleic acid slope	−23 1	2 7	−8 3	1 6	$p<0$ 005
FA linoleic acid slope	−5 8	3 1	2 8	1 9	$p<0$ 05

noleic acid in plasma TG were of similar agnitude in the two groups (Table 2) Peak FA concentrations did not differ in the two oups after the single fat injection The FFA eaks were minute and of no constant appear

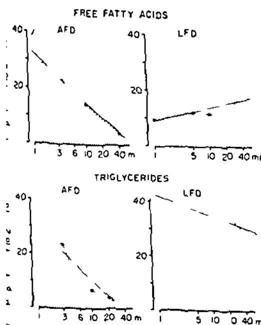


Fig 2 Single injection Proportion of linoleic acid (18) 1-6) in FFA and TG related to time after injection (time expressed on a logarithmic scale) in one AFD infant and in one LFD inf nt Dotted areas indicate the preinjectional levels Regression l nes drawn ndicate FFA and TG linoleic acid slopes

ance during the observation period of 40 min The peaks of FFA linoleic acid did not differ in amount in the two groups but did appear at different times In AFD FFA linoleic acid peak appeared early and in LFD late after the injection This difference between the two groups is apparent from the FFA linoleic acid content within 10 min after the injection (Table 2) Representative curves to illustrate the changes in plasma FFA and triglyceride linoleic acid content at various times after the single injection of Intralipid® are shown both for AFD and LFD in Fig 2 In the AFD infant linoleic acid (18 2) showed a rapid decrease in both TG and FFA The LFD infant on the other hand revealed a slower reduction in plasma TG linoleic acid and a gradual build up of FFA linoleic acid In LFD the increase in FFA linoleic acid still proceeded at the time when linoleic acid was already cleared from plasma FFA of the AFD infants The slope of the regression line (cf Fig 2) is an expression of the rate of change in linoleic acid (18 2) proportion and is here called linoleic acid slope The linoleic acid slope for plasma TG and FFA was calculated for each infant The linoleic acid slope for plasma TG differed in the two groups ($p<0$ 005) as did the linoleic acid slope for plasma FFA ($p<0$ 05) (Table 2) There was a correlation between linoleic acid

Table 1 *Fatty acid distribution in Intralipid® triglycerides*

Fatty acids	Per cent by weight (%)
16 0 palmitic acid	10
18 0 stearic acid	5
18 1(n 9) oleic acid	25
18 2(n-6) linoleic acid	50
18 3(n 3) linoleic acid	10

Figures stand for number of carbon atoms and number of double bonds (n) stand for number of carbon atoms from the methyl end to the first double bond

whether the observed difference in lipid metabolism in AFD and LFD infants was related to release of FFA from plasma TG or to elimination of FFA from the blood stream

MATERIALS AND METHODS

Clinical material

Low birth weight infants (<2 500 g) were studied Only infants in whom the obstetrical record gave reliable data on gestational age in agreement with the findings on physical examination were included In 3 infants either single or repeated injections were performed while 11 infants had both studies done Birth weight and gestational age of the infants are presented in the diagram (2) in Fig 1 Two pairs of identical twins were included in the series Within both pairs a weight difference existed In one pair both infants were AFD in the other both were LFD All infants were studied in the fasting state 2–13 hours after birth No food was given before or during the study In one pair of twins (the LFD pair) only the procedure of repeated injections was performed

Procedures

Intralipid® was administered either through an umbilical vein (tip of catheter in v cava inferior) or umbilical artery (tip of catheter in aorta at the level of the diaphragm) since it is known that the mode of administration is not critical for substances with a low turnover rate due to the rapid dilution (9) (The total blood volume of a 2 kg infant has circulated four times through the body in one minute) Position of catheter was controlled by a TV fluoroscopy

The single injection of Intralipid® was given at a dose of 0 10 g/kg b w Blood samples were drawn before 1 5 10 15 20 and 40 min after the single injection Blood samples (0 8–1 0 ml) were drawn from the umbilical catheter after careful rinsing to avoid admixture with injected fat

At the procedure of repeated injections these were given hourly for 6–8 hours with a first dose of 0 10 g and the following doses of 0 15 g/kg b w In this procedure the samples were drawn 40 min after each injection for the measurement of turbidity in time for the next injection Turbidity >50 units was set as a limit for further Intralipid® injections Occasional samples had to be omitted due to technical difficulties or for clinical reasons

Chemical methods

To allow the use of small plasma samples concentrations of TG and FFA were determined from the same gas–liquid chromatograms (GLC) (6) utilizing an internal standard TG and FFA were isolated by thin layer chromatography (TLC) on silica gel and TG and FFA spots scraped into glass tubes with teflon caps Before the transmethylation by sulphuric acid in dry methanol a known amount of methyl heptadecanoate was added to each tube GLC of methyl esters and identification of peaks were performed as described earlier (7 8) Peak areas on the chromatogram were estimated and compared with that of the known methyl heptadecanoate

A good correlation ($r=0\ 99$ $n=70$) was found between the TG concentrations determined by GLC and by a spectrophotometrical method (1) Mean per cent difference between the two methods was 7 1±4 3% (6)

RESULTS

Single injection

Before fat administration plasma TG and FFA revealed a great variation (range 15–110 mg/100 ml and 6–35 mg/100 ml respectively) although there was no difference between the AFD and LFD groups (Table 2) Before fat administration the relative content of linoleic acid was higher in LFD both in TG and FFA ($p<0\ 005$)

After the single Intralipid injection of 0 10 g/kg b w all infants both those in the AFD and LFD groups revealed increased TG The peaks of plasma TG as well as proportions of

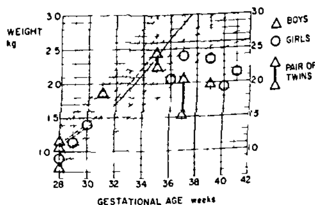


Fig 1 Distribution of patient series according to birth weight and gestational age (2)

Table 2 *Single injection Various plasma lipid data before and after a single injection of Intralipid® (0 10 g fat/kg b w /h) in 8 AFD and 5 LFD infants*

	AFD (n=8)		LFD (n=5)		Significance of difference
	Mean	S E M	Mean	S E M	
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TG linoleic acid slope	−23 1	2 7	−8 3	1 6	p<0 005
FFA linoleic acid slope	−5 8	3 1	2 8	1 9	p<0 05

linoleic acid in plasma TG were of similar magnitude in the two groups (Table 2) Peak FFA concentrations did not differ in the two groups after the single fat injection The FFA peaks were minute and of no constant appearance during the observation period of 40 min The peaks of FFA linoleic acid did not differ in amount in the two groups but did appear at different times In AFD FFA linoleic acid peak appeared early and in LFD late after the injection This difference between the two groups is apparent from the FFA linoleic acid content within 10 min after the injection (Table 2) Representative curves to illustrate the changes in plasma FFA and triglyceride linoleic acid content at various times after the single injection of Intralipid® are shown both for AFD and LFD in Fig 2 In the AFD infant linoleic acid (18 2) showed a rapid decrease in both TG and FFA The LFD infant on the other hand revealed a slower reduction in plasma TG linoleic acid and a gradual build up of FFA linoleic acid In LFD the increase in FFA linoleic acid still proceeded at the time when linoleic acid was already cleared from plasma FFA of the AFD infants The slope of the regression line (cf Fig 2) is an expression of the rate of change in linoleic acid (18 2) proportion and is here called linoleic acid slope The linoleic acid slope for plasma TG and FFA was calculated for each infant The linoleic acid slope for plasma TG differed in the two groups (p<0 005) as did the linoleic acid slope for plasma FFA (p<0 05) (Table 2) There was a correlation between linoleic acid

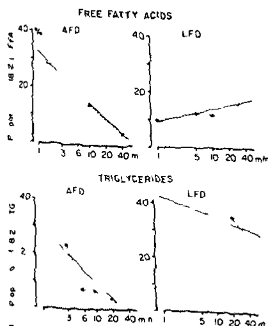


Fig 2 Single injection Proportion of linoleic acid (18 2, n-6) in FFA and TG related to time after injection (time expressed on a logarithmic scale) in one AFD infant and in one LFD infant Dotted areas indicate the preinjectional levels Regression lines drawn indicate FFA and TG linoleic acid slopes

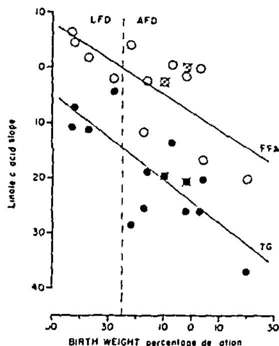


Fig 3 Single injection TG linoleic acid slopes and FFA linoleic acid slopes respectively as a function of percentage deviation from mean birth weight for gestational age ●=TG ○=FFA Circles with crosses indicate pair of twins

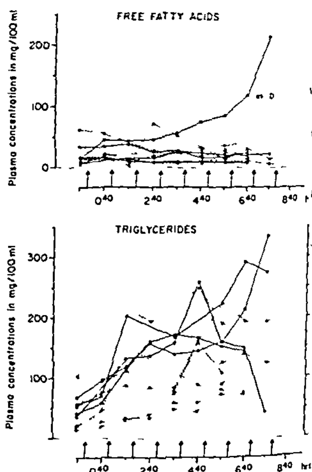


Fig 4 Repeated injections TG and FFA levels in mg/100 ml plasma during repeated injections of Intralipid in 7 AFD and 4 LFD infants ___ LFD AFD Arrows indicate time of Intralipid injections

slope and deviation in birth weight both in TG ($r=-0.79$ $p<0.01$) and in FFA ($r=-0.74$ $p<0.01$) (Table 2 Fig 3) These correlations were not influenced if the pair of twins were excluded The degree of deviation in birth weight explained in TG 62% and in FFA 54% of the correlations Multiple regression analyses with two additional X variables gestational age of infant and time from birth to injection were performed These two variables were of secondary and tertiary importance respectively For TG the time from birth to injection was the second factor and for FFA gestational age was second factor The addition of these two X variables did not however increase the precision of the prediction of the multiple regression analysis to any statistically significant degree Thus the deviation in birth weight was the primary factor in explaining the regression

Table 3 *Repeated injections TG and FFA plasma concentrations and linoleic acid proportions (18 2%) in 7 AFD and 4 LFD infants Mean of 5th–7th postinjectional values*

	AFD ($n=7$)		LFD ($n=4$)		Significance of differences
	Mean	S E M	Mean	S E M	
TG mg/100 ml	133	20	194	26	$p<0.05$
FFA mg/100 ml	21	3	36	19	N S
18 2% in TG	31	2	35	2	N S
18 2% in FFA	22	2	25	2	N S

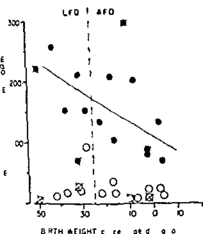


Fig 5 Repeated injections TG and FFA levels (mean of the levels after the 5th to the 7th injections) as a function of percentage deviation from mean birth weight for gestational age ● = TG O = FFA Circles with crosses indicate two pairs of twins

Repeated injections

After (40 min) each hourly injection of 0 15 g fat/kg b w plasma TG increased in all infants (Fig 4) When mean plasma TG (in samples taken 40 min after the 4th 5th and 6th injection) for each infant was calculated and group means were compared (Table 3) TG was higher ($p<0\ 05$) in LFD Plasma FFA did not increase except in one (case D) (Fig 4) The linoleic acid (18 2) content in FFA and TG did not differ in the two groups (Table 3)

In the combined groups of LFD and AFD infants mean plasma TG correlated ($r=-0\ 65$ $p<0\ 05$) with percentage deviation of birth weight (Fig 5) In this calculation values for the two pairs of twins were not included While no correlation was found between mean plasma FFA and birth weight deviation there was a correlation ($r=-0\ 68$ $p<0\ 01$) between sum of TG and FFA with birth weight deviation

Twins

Two pairs of identical twins were studied In one pair both twins belonged to the AFD group and in the other pair both were LFD (Fig 1) Within both pairs a weight difference existed The linoleic acid slope for plasma TG and FFA after single injection did not differ in the AFD twin pair as compared with those of the whole series (cf Fig 3) After repeated

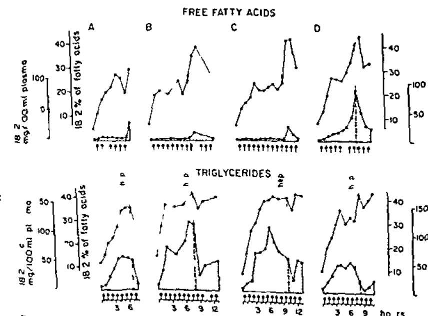


Fig 6 Heparin injection in 4 infants Dotted areas mark the concentration of linoleic acid (18 2 n-6) in triglycerides and free fatty acids respectively — Proportion of linoleic acid Arrows indicate time for Intralipid® injections Vertical line marks heparin injection

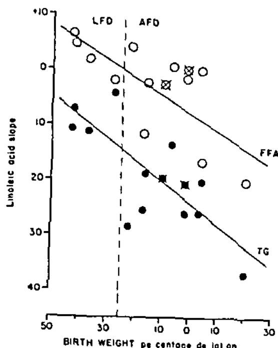


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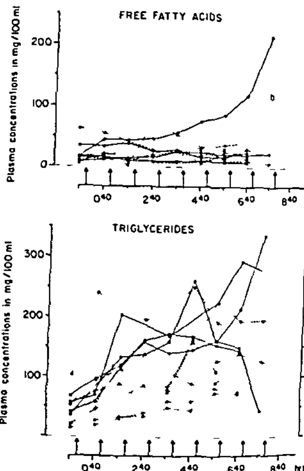


Fig 4 Repeated injections TG and FFA levels in mg/100 ml plasma during repeated injections of Intralipid in 7 AFD and 4 LFD infants —— LFD AFD Arrows indicate time of Intralipid injections

slope and deviation in birth weight both in TG ($r=-0\ 79$ $p<0\ 01$) and in FFA ($r=-0\ 74$ $p<0\ 01$) (Table 2 Fig 3) These correlations were not influenced if the pair of twins were excluded The degree of deviation in birth weight explained in TG 62% and in FFA 54% of the correlations Multiple regression analyses with two additional X variables gestational age of infant and time from birth to injection were performed These two variables were of secondary and tertiary importance respectively For TG the time from birth to injection was the second factor and for FFA gestational age was second factor The addition of these two X variables did not however increase the precision of the prediction of the multiple regression analysis to any statistically significant degree Thus the deviation in birth weight was the primary factor in explaining the regression

Table 3 *Repeated injections TG and FFA plasma concentrations and linoleic acid proportions (18 2 %) in 7 AFD and 4 LFD infants Mean of 5th–7th postinjectional values*

	AFD ($n=7$)		LFD ($n=4$)		Significance of differences
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ACKNOWLEDGEMENTS

e skilful technical assistance of Mrs Anita Fae is grate ly acknowledged Multiple regression analyses were formed by Thorvald Landstrom (B Sc) This study s supported by grants from Semper Fund for Nutri nal Research AB Vitrum and Fels Research Institute the Study of Human Development

REFERENCES

Carlson L A Determination of serum glycerides *Acta Soc Med Upsal 64* 08 1959

2 Engström L Karlberg P & Selstam U Swedish standard curves for the weights and lengths of newborn infants In preparation

3 Gustafson A Kjellmer I Olegård R & Victorin L Nutrition in low birth weight infants I Intravenous injection of fat emulsion *Acta Paediatr Scand 61* 149 1972

4 Gustafson A Kjellmer I Olegård R & Victorin L Nutrition in low birth weight infants II Repeated injections of fat emulsion *Acta Paediatr Scand 63* 177 1974

5 Hallberg D Elimination of exogenous lipids from the blood stream *Acta Physiol Scand 65* Suppl 254 1965

6 Olegård R Essential fatty acids in the early postnatal period Submitted for publication

7 Olegård R & Svennerholm L Fatty acid composition of plasma and red blood cell phosphoglycerides in three months-old infants *Acta Paediatr Scand 60* 505 1971

8 Svennerholm L Distribution and fatty acid composition of phosphoglycerides in normal human brain *J Lipid Res* 9 570 1968

9 Wallgren G Hanson J S Tobakin B S Räihä N & Vapaavuori E Hemodynamic findings in premature infant with and without respiratory distress *Acta Paediatr Scand* Suppl 179 p 69 1967

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injections, on the other hand the light twin in each pair accumulated more TG than the heavier twin (cf Fig 5)

Heparin administration

Heparin was administered (50–100 units/kg b w) after 6–8 fat injections in 4 LFD infants The effect on plasma concentration and linoleic acid (*18 2*) content in plasma TG and FFA are shown in Fig 6 In 3 (A–C) plasma TG increased with each Intralipid® injection Plasma TG linoleic acid content increased successively with plasma TG concentration while plasma FFA were low After the heparin injection plasma TG and plasma TG linoleic acid (*18 2*) content decreased in all cases Simultaneously plasma FFA and plasma FFA linoleic acid (*18 2*) increased in 3 cases (A–C) In case D plasma TG declined and plasma FFA and plasma FFA linoleic acid content increased already before the heparin injection After the heparin injection plasma FFA and plasma FFA linoleic acid content decreased markedly in case D

DISCUSSION

After the single injection of Intralipid® the removal of the administered triglycerides could be expressed as the TG linoleic acid slope A steeper slope i e a slope with a more negative value would indicate a faster removal of exogenous TG In low birth weight infants who were light for date (LFD) this expression for removal was reduced as compared with those appropriate for date (AFD) Furthermore in the combined series of AFD and LFD infants TG linoleic acid slope related to deviation in birth weight (Fig 3) indicating that the elimination of administered TG was slower with more severe underweight of the infant

The corresponding FFA linoleic acid slope would be influenced by not only (*a*) release of FFA from Intralipid® but also (*b*) the subsequent uptake of released FFA (in muscles liver and adipose tissue) and furthermore by (*c*) FFA released from adipose tissue In the newborn baby with low adipose tissue stores of linoleic acid (6) only the two first factors are pertinent The initial increase (within 10 min) of FFA linoleic acid was appreciable in AFD but low in LFD infants (Table 2) In AFD infants FFA linoleic acid slope was negative (cf Fig 2) indicating a sufficient elimination of FFA from plasma In LFD infants FFA linoleic acid slope was positive (cf Fig 2) indicating that FFA were not eliminated from plasma in proportion to their release from Intralipid® In individual data a negative FFA linoleic acid slope was obtained in 5 AFD and in one LFD infant while positive slopes were estimated in 3 LFD and 2 AFD infants (cf Fig 3) This would indicate a metabolic overlapping between the two clinical groups of AFD and LFD infants

Thus the total series of LBW infants should be looked upon as a continuum since the combined series of AFD and LFD infants revealed a correlation ($r=-0\ 74$ $p<0\ 01$) between FFA linoleic slope and birth weight deviation A similar impression of continuum was revealed from the correlation between TG linoleic acid slope and birth weight deviation ($r=-0\ 79$ $p<0\ 01$)

After repeated hourly injections of Intralipid® the data on release and elimination of FFA linoleic acid were in agreement with those obtained from the studies on a single injection In LFD infants the accumulation of exogenous TG increased with more severe underweight and FFA released from exogenous fat increased gradually Thus it appeared to be a balance between release and elimination of FFA but at a retarded speed In one LFD infant (case D) this balance was not apparent (cf Fig 4) as the released FFA (FFA linoleic acid) appeared to accumulate

In two pairs of twins the pattern of exogenous TG removal expected from their birth weight (in relation to gestational age) was not followed Independent of their clinical classification as AFD or LFD the heavier twin in each pair had an adequate TG elimination

while the lighter twin showed accumulation A possible explanation might be that when weight differences occur within a pair, the lighter twin is metabolically light for-date in relation to its control twin independent of its place in a growth–gestational age diagram

Heparin caused a stimulation of the release of FFA from Intralipid® TG as judged from the appearance of FFA linoleic acid Further more the release of FFA from Intralipid® TG was stimulated more than the elimination from plasma of released FFA In one case heparin appeared to have an effect also on the elimina tion of released FFA (FFA linoleic acid)

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The skilful technical assistance of Mrs Anita Fae is grate fully acknowledged Multiple regression analyses were performed by Thorvald Landstrom (B Sc) This study was supported by grants from Semper Fund for Nutri tional Research AB Vitrum and Fels Research Institute for the Study of Human Development

REFERENCES

1 Carlson L A Determination of serum glycerides *Acta Soc Med Upsal 64* 208 1959
2 Engstrom L Karlberg P & Selstam U Swedish standard curves for the weights and lengths of newborn infants In preparation
3 Gustafson A Kjellmer I Olegård R & Victorin L Nutrition in low birth weight infants I Intrave nous injection of fat emulsion *Acta Paediatr Scand 61* 149 197
4 Gustafson A Kjellmer I Olegård R & Victorin L Nutrition in low birth weight infants II Repeated injections of fat emulsion *Acta Paediatr Scand 63* 177 1974
5 Hallberg D Elimination of exogenous lipids from the blood stream *Acta Physiol Scand 65* Suppl 254 1965
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8 Svennerholm L Distribution and fatty acid composi tion of phosphoglycerides in normal human brain *J Lipid Res 9* 570 1968
9 Wallgren G Hanson J S Tobakin B S Raihä N & Vapaavuori E Hemodynamic findings in prema ture infant with and without respiratory distress *Acta Paediatr Scand* Suppl 179 p 69 1967

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MUSCULAR EXERTION
A TEST OF PITUITARY FUNCTION IN CHILDREN

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A CACCAMO and G CHIUMELLO

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ABSTRACT Garlaschi C Del Guercio M J, Di Natale B Caccamo A and Chiumello G (Department of Paediatrics University of Milan Italy) Muscular exertion a test of pituitary function in children Acta Paediatr Scand 64 752 1975 —The *elevated level of growth hormone after moderate standardized physical exercise* was compared with that induced by intravenous arginine infusion and by insulin induced hypoglycemia in children with normal pituitary function Tests were performed on 49 prepubertal children (34 boys and 15 girls) in 42 cases the increase was significant for all three tests in 5 cases the response was minimal after insulin stimulation but normal after arginine and physical exertion in 1 case arginine produced no response but the other tests were positive in 1 case there was a response to arginine but none to insulin or physical exertion The results indicate that frequently more than one test is necessary for the diagnosis of normal pituitary function physical exertion being a physiologic test is simple to perform acceptable to the children and without side effects It appears the test of first choice because it can be used in patients seen ambulatorily other tests being performed in case of doubt or negative response

KEY WORDS Growth hormone physical exertion pituitary function

The physiologic and pharmacologic stimuli which cause a rise in the plasma level of the growth hormone (GH) are numerous Among the physiological factors severe physical exertion without changes in the blood sugar level has long been known to cause a significant increase in the plasma GH (6 7 11) the mechanism underlying this increase in GH level is a matter for discussion but it is clear that the sympathetic nervous system plays an important role as demonstrated by the effect of adrenergic blockers on the plasma GH response after physical exercise (5) Recently physical exercise has been proposed as a screening for growth hormone release a moderate exercise could represent a physiologic stimulus better than other pharmacologic stimuli (2)

The present paper reports changes in plasma GH after mild physical exertion standardized by the use of a cycle ergometer the responses were compared with those induced by intravenous infusion of arginine and by insulin induced hypoglycemia in children with normal pituitary function

MATERIAL AND METHODS

Tests were performed on 49 prepubertal children (34 boys and 15 girls) aged 8–11 years and admitted to the metabolic Unit on account of retardation of growth and development

Thyroid function was evaluated by estimation of T_4 and T_3 levels in basal conditions and after stimulation with TSH the hypothalamo hyphyseo adrenal system by estimation of the plasma cortisol urinary 17–OH corticosteroids and 17 ketosteroids in basal conditions and following administration of metyrapone and alpha 1 24 corticotropin Zn plasma LH urinary gonadotropins

The GH levels were determined in basal conditions following intravenous infusion of arginine (ATT) and insulin (ITT) and after physical exercise

Table I *Effect of physical exercise, i v infusion of arginine (A T T) and insulin (I T T) on the plasma GH levels (mean±S E M)*

	0 min	30 min	60 min	90 min	Peak value
ATT HGH ng/ml	2 6±0 3	9 4±1 3	14 6±2 1	6 7±1 9	16 9±2 *
	0 min	20 min	40 min	60 min	
ITT HGH ng/ml	2 7±0 7	7 9±1 9	11 0±1 4	9 8±1 3	14 8±2 2
	0 min	10 min	12 min	15 min	
Exercise test HGH ng ml	3 1±0 5	12 3±1 8	14 5±2 2	14 2±2 3	15 6±2 3

p value<0 05   *p* value<0 01

L arginine monochloride was administered by intravenous infusion over a period of 30 min at a dosage of 0 5 g/kg body weight dissolved in *hypotonic Ringer solution* insulin was given by rapid intravenous injection at a dosage of 0 1 U/kg body weight diluted in 2 ml of physiological saline The physical exertion was *standardized* by using a cycle ergometer (Monark Stockholm) for 10 min at 150 or 300 kpm according to statural age (under or over 8 years) Among the patients with a statural age below 8 years as there was no significant difference in GH response between those who had performed the physical exertion at 150 kp/min (about 50% of the maximal work ing capacity) and those who made the exercise at 50 kpm/min (about 75% of the maximal working capacity) it has been decided to perform the lower dosage of exercise

For the patients with a statural age over the 8 years the same percentage of maximal working capacity (about 50 which responds to 300 kpm/min) has been chosen

The maximal working capacity calculated on the basis of a linear relationship between heart rate and work load corresponds to an assumed heart rate of 200 min and 05 m n for a statural age respectively lower and higher than 8 years (3)

The three tests were performed at intervals of 48 hours After an overnight fast venous catheterization was performed at 8 30 a m isotonic s line solution was infused for 0 min and a sample was coll cted (time 0 min) immediately before the administration of arginine or insulin or before the physical exercise Blood samples were taken 30 60 and 90 minutes after the start of the arginine infu ion 0 40 and 60 min after the administration of insulin nd 10 12 and 15 min after the start of the physical exercise

Blood ugar was determined by means of an autoanalyzer and plasma GH levels by radioimmunoa say (10) The heart rate and the blood pressure were checked before during and after the exercise

RESULTS

On the basis of the results obtained a diagnosis of retardation of growth and development with normal endocrine function was made in all 49 patients

The intravenous arginine infusion was well tolerated in all the patients but the insulin stimulation test had to be stopped in 10 patients owing to signs of hypoglycemia The physical exercise was well tolerated by all the children standardized as indicated above it was well within their capability and was certainly better accepted than the other tests In all the patients the blood pressure and the heart rate remained within physiologic limits and there were no changes in the blood sugar during the period of exertion

The GH levels recorded during the three tests are shown in Table I In 42 patients the increase was significant for all the three tests in 5 patients the response was minimal (an increase below 5 ng/ml) after the insulin stimulation but normal after arginine and the physical exercise in one patient arginine produced no response but the other two tests were positive in one patient there was a response to arginine but not to insulin and exercise

The tests which gave negative results were subsequently repeated and normal results were obtained

DISCUSSION

A test of pituitary function should be easily reproducible and well tolerated

The most used tests are arginine stimulation and insulin induced hypoglycemia Although the former is well tolerated by the patient and causes no side effects the interpretation of the results may be difficult as the response may vary with age and with the degree of sexual maturity (12) The insulin test demands ex

treme care of the patient because of the risk of a hypoglycemic shock even though the patient s age and sexual development may influence the GH response, it is however generally agreed that the test reflects the pituitary function(4)

It has been known for many years that intense physical exertion stimulates GH secretion (6 11) though the mechanism by which physical exercise causes an increase in GH secretion is not known While it is questionable whether beta adrenergic mechanism is the cause of growth hormone release it is certain that alpha adrenergic blockade inhibits the response and beta adrenergic blockade increases it (5) Physical exertion has been proposed as a simple screening procedure for evaluating short stature in children by step climbing and brisk walking or by pedalling for 10 minutes on a bicycle increasing the endurance as far as the child could reasonably tolerate the effort (1 8 9)

Standardized mild physical exertion provides a plasma GH stimulus comparable to that of arginine or insulin induced hypoglycemia The results obtained in the present series indicate that in many patients more than one test is necessary to confirm the diagnosis of a normal pituitary function and that it is sometimes necessary to repeat a test A good response excludes subnormal pituitary somatotropic function but failure to response must be carefully interpreted in the light of the clinical findings before reaching a definite conclusion

Physical exertion is a physiological test which is simple to perform and more acceptable to children than the other tests owing to its shorter duration moreover it causes no side effects such as are often observed in insulin induced hypoglycemia

On the basis of these findings exertion appears to be the primary test of choice in the evaluation of growth retarded children in ambulatory practice as a screening procedure even though other tests must be performed in case of doubt or in case of negative response

Further studies are currently in progress concerning the effects of age and sexual development on the GH response to exertion

REFERENCES

1 Buckler J M H Plasma growth hormone response to exercise as diagnostic aid *Arch Dis Child* 48 565 1973
2 — Exercise as screening test for growth hormone release *Acta Endocrinol* 69 219 1972
3 Cerretelli P *Fisiologia del lavoro e dello sport* SEU Milan 1973 p 266
4 Frasier S D Hilburn J M & Smith F G Effect of adolescence on the serum growth hormone response to hypoglycaemia *J Pediatr* 77 465 1970
5 Hansen A P The effect of adrenergic receptor blockade on the exercise induced serum growth hormone rise in normals and juvenile diabetics *J Clin Endocrinol Metab* 33 807 1971
6 Hartog M Havel R J Copinschi G & Earll J L The relationship between changes in serum levels of growth hormone and mobilization of fat during exercise in man *Q J Exp Physiol Cog Med Sci* 52 86 1967
7 Hunter W M Fonseka C C & Passmore R The role of growth hormone in the mobilization of fuel for muscular exercise *Q J Exp Psychol* 50 406 1965
8 Keenan B S Killmer C B & Sode J Growth hormone response to exercise A test of pituitary function in children Pediatrics 50 760 1972
9 Lacey K A Hewison A & Parkin J M Exercise as a screening test for growth hormone deficiency in children *Arch Dis Child* 48 508 1973
10 Morgan C R Human growth hormone immunoassay two antibody methods using I[125] tracer *Proc Soc Exp Biol Med* 121 62 1968
11 Roth J Glick S M Yalow S & Berson S A Secretion of human growth hormone physiologic and experimental modification *Metabolism* 12 577 1963
12 Sperling M A Kenny F M & Drash A L Arginine induced growth hormone response in children effect of age and puberty *J Pediatr* 77 462 1970

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SUBACUTE NECROTIZING ENCEPHALOMYELOPATHY

Clinical Ultrastructural Biochemical and Therapeutic Studies in an Infant

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From the Departments of Pædiatrics and of Medical Cell Biology University of Munster Munster and from the Institute of Human Genetics Medical School Lubeck BRD

ABSTRACT Grobe H v Bassewitz, D B Dominick H Chr and *Pfeiffer R A* (Departments of Paediatrics and of Medical Cell Biology University of Munster Munster and Institute of Human Genetics Medical School Lubeck BRD) Subacute necrotizing encephalomyelopathy Clinical ultrastructural biochemical and therapeutic studies in an infant. Acta Paediatr Scand 64 755 1975.—Subacute necrotizing encephalomyelopathy (SNE) has been observed in an infant with regressing psychomotor development The concentrations of alanine pyruvate and lactate were increased in the serum and blood as well as in the cerebrospinal fluid Pyruvate carboxylase activity was reduced in the liver tissue An inhibitor of thiamine pyrophosphate-ATP phosphotransferase was present in the urine Thiamine treatment was followed by a decrease of serum alanine and blood pyruvate and lactate but there was no clinical improvement during a period of 17 months Ultrastructural investigations revealed high glycogen levels in liver tissue and skeletal muscle These findings contrast with decreased gluconeogenesis which is suggested by the diminished pyruvate carboxylase activity *Therefore it is concluded that reduced hepatic pyruvate carboxylase activity is not the primary cause of SNE*

KEY WORDS Subacute necrotizing encephalomyelopathy pyruvate carboxylase thiamine treatment electron microscopy glycogen

he first description of subactue necrotizing ıcephalomyelopathy (SNE) is attributed to eigh (18) This condition is characterized by eurological abnormalities such as muscular)potonia lack of coordination of the ex *emities oculomotor palsy nystagmus* vis al disturbances and regressing psychomotor evelopment (14) The lethal disorder starts rior to the end of the second year of life (24) ut in rare instances it has been observed in adolescents and even in adults (27) Anatomi cal findings include symmetrical lesions of the basal ganglia brain stem cerebellum and spinal cord (14 22) The familial incidence of the disorder suggests an autosomal recessive mode of inheritance

Constant findings also include increased *levels* of lactate and pyruvate in the blood and of alanine in the serum (2 8 12 22) In some cases however the lactate concentration was normal (7 21 23) Attempts at treatment have been made with lipoic acid (2 8 10) biotin (8) *and thiamine* or thiamine derivatives (8 10 13 22–24) but it is difficult to evaluate the efficacy of any of these compounds because spontaneous remissions in SNE have also been reported (24)

This paper w pre ented in part at the tenth Arbeitsagung fur Pad trische Forschung Marburg, 18–19

Table I *Lactate pyruvate and alanine levels in blood/serum and in cerebrospinal fluid (CFS)*

The values are given in mg per 100 ml

	Lactate	Pyruvate	Alanine
Blood/serum	28 8	2 34	12 1
Normal range	9–16	0 36–0 59	1 84–4 40
(ref)	(17)	(16)	
CSF	52 5	4 35	1 02
Normal range	7 5–21 7	0 4–0 7	0 10–0 40
(ref)	(20)	(9)	(6)

CASE REPORT

A K a girl born January 9 1971 was the only child of unrelated healthy parents Pregnancy and labour were reported to have been normal The birth weight was 2 740 g the length 52 cm The neonatal period was uneventful At the age of 6 weeks the girl was able to lift her head an ability which she lost during her later life At about 10–12 weeks the mother noticed that the infant was unable to fixate objects At the age of 7 months she was admitted to a hospital and a severe cerebral disorder of unknown etiology was diagnosed

The child was first admitted to our hospital at the age of 14 months On physical examination she appeared extremely mentally retarded She weighed 8 300 g was 70 cm tall and had a head circumference of 42 5 cm which was below the third percentile for her age The liver and spleen were not enlarged She was not able to fix her eyes upon any object and showed searching eye movements No spontaneous movements of the extremities were observed but the child occassionally had myoclonic jerks Muscle tone and deep tendon reflexes were increased Both plantar responses were extensor No Moro response or asymmetric tonic neck reflexes were elicited The child was unable to sit and lacked head control

Laboratory findings Blood counts were normal Sodium 143 mEq/l potassium 4 3 mEq/l chloride 110 mEq/l blood urea nitrogen 20 mg/100 ml PBI 10 7 μg/100 ml serum fasting blood glucose 60 mg/100 ml pH 7 39 P_{CO_2} 40 mmHg standard bicarbonate 23 5 mEq/l base excess −0 5 mEq/l

X ray of the skull microcephaly without premature synostosis no intracranial calcifications EEG multifocal hypersynchronous activity PEG enlargement of the lateral ventricles Lumbar puncture clear fluid 8/3 cells/mm³ and 17 mg total protein/100 ml Funduscopy bilateral partial atrophy of the optic nerve

Biochemical and Ultrastructural Investigations

Methods

Analyses of amino acids were performed by ion exchange chromatography by the method of Spackman et al (28) Blood lactate was measured enzymatically by the method of Hohorst (11) pyruvate was determined enzymatical by the method of Czok & Lamprecht (5)

Liver pyruvate carboxylase activity determination wa based on the method described by Utter & Keech (31 The method for testing the urine for the inhibitor o the thiaminepyrophosphate ATP phosphotransferase ha been described by Cooper et al (3) Biopsy specimen from the liver and skeletal muscle were processed fo fine structural investigations as described elsewhere (1)

Results

The results of the quantitative analyses of alanine in the serum and the cerebrospinal fluid and of lactate and pyruvate in the blood and the cerebrospinal fluid are summarized in Table I There was a marked increase in the concentration of alanine in the serum and the CSF Al the other amino acids were within normal limits The concentrations of pyruvate and lactate in the blood and the CSF were above the normal range After an oral intake of 150 mg l alanine per kg bodyweight no increase of blood glucose was noted

The specific activity of the pyruvate carboxylase in liver tissue was 0 054 μmols/min/g wet weight and was very low compared with controls The phosphoenolpyruvate carboxykinase activity (3 7 μmole/min/g wet weight) was quite normal

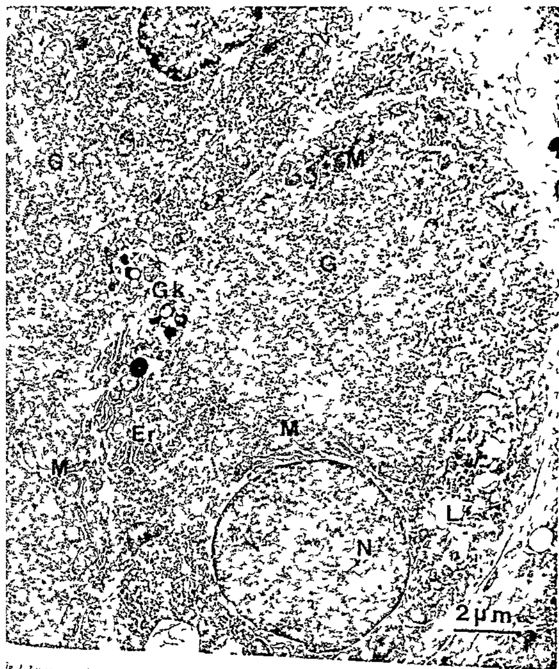
In the urine an inhibitor of the thiaminepyrophosphate ATP phosphotransferase was present

The electron microscope investigations showed a well preserved lobular architecture of the liver The cells of the portal tracts did not exhibit any alteration on the ultrastructural level The parenchymal cells were arranged in plates The volume of the hepatocytes was increased due to an excessive glycogen accumulation (Fig 1) Fatty transformation was visible in a small droplet pattern No specific alterations of the mitochondria or the endoplasmic reticulum were found but these organelles were predominantly located at the cell periphery The Golgi complex the lysosomes and the bile canaliculi had a normal appearance

The ultrastructural arrangement of the skeletal muscle cells was normal However an increased amount of glycogen granules was found between the myofibrils near the cell membrane and around the nucleus Lipid droplets with an average diameter of 0 5 μm were scattered between the myofibrils (Fig 2) In several muscle cells concentric laminated bodies were observed These structures consisted of concentrically arranged membranes surrounding glycogen granules Depending on the section angle these bodies appear circular or ellipsoid in shape (Fig 2)

TREATMENT

At the age of 18 months the child was given thiaminechloride hydrochloride (vitamin B1) in a dosage up to 1 8 g per day and l aspartate up to 2 1 g per day The concentrations of the blood pyruvate and lactate of the serum alanine and of the urine alanine all decreased (Fig 3) However the concentration of pyruvate did not decrease to normal ... the lactate constantly re



ig 1 Liver parenchymal cells Due to the high glycogen) content the parenchymal cell volume is enlarged itochondria (M) and rough endoplasmic reticulum (Er) are located in the cell periphery Nucleus (N) Lipid droplets (L) Bile canaliculus (Gk) ×8500

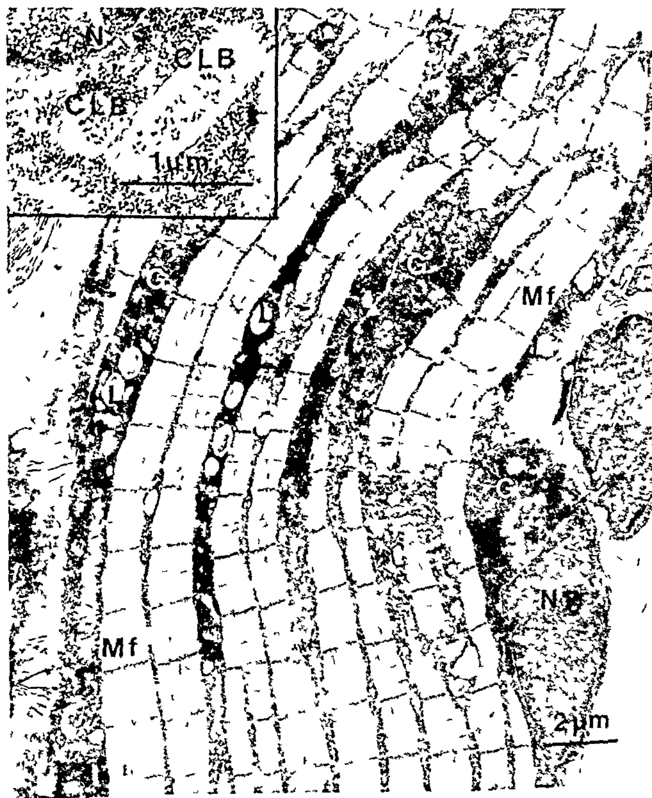


Fig 2 Skeletal muscle cell A high glycogen (*G*) content can be demonstrated between the myofilaments (*Mf*) and near the nucleus (*N*) Lipid droplets (*L*) ×9000 *Inset* Concentric laminated bodies (*CLB*) Nucleus (*N*) ×30000

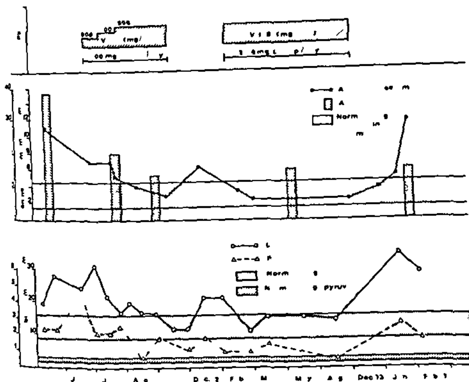


Fig 3 Therapeutic effect of thiamine (Vit B_1) and aspartate on pyruvate lactate and alanine levels in blood/serum and on alanine excretion in urine in one child with subacute necrotizing encephalomyelopathy

tained near the upper normal limit The temporary suspension of thiamine administration was followed by increase of the biochemical parameters Pyruvate and lactate in the blood and alanine in the serum both decreased again after resumption of the thiamine therapy

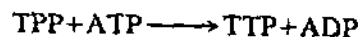
Despite the biochemical improvement the clinical condition of the child showed no improvement during a period of 17 months On the contrary the girl developed progressive neurological impairment There were hyperactive deep tendon reflexes and spontaneous bilateral Babinski signs The pupils reacted slowly to light The musculature was generally weak She became drowsy and sometimes fell into a stupor for periods of 2 to 3 days and had feeding difficulties The frequency of seizures was unaltered At the age of 3 years the ineffective therapy was suspended being once more followed by an increase of pyruvate lactate and alanine in the blood and serum

DISCUSSION

The diagnosis of SNE was based upon the increased concentrations of alanine pyruvate and lactate in the serum blood and CSF and also upon the reduced activity of pyruvate carboxylase It was further substantiated by the presence of the inhibitor of thiamine pyrophosphate ATP phosphotransferase in the urine

In order to explain the clinical and biochemical findings in SNE two different mechanisms have been suggested

1 The inhibition of thiaminepyrophosphate ATP phosphotransferase since an inhibition *factor was found in the blood urine and CSF* which is not present in healthy persons (3 23) This enzyme catalyses the reaction



$$TPP+ATP \longrightarrow TTP+ADP$$

A deficiency of thiaminetriphosphate (TTP) could be particularly harmful to the CNS (4)

2 The decreased pyruvate carboxylase activity may cause a defective gluconeogenesis (12) Decreased enzyme activity is also followed by an increase of blood pyruvate blood lactate and plasma alanine (Fig 4)

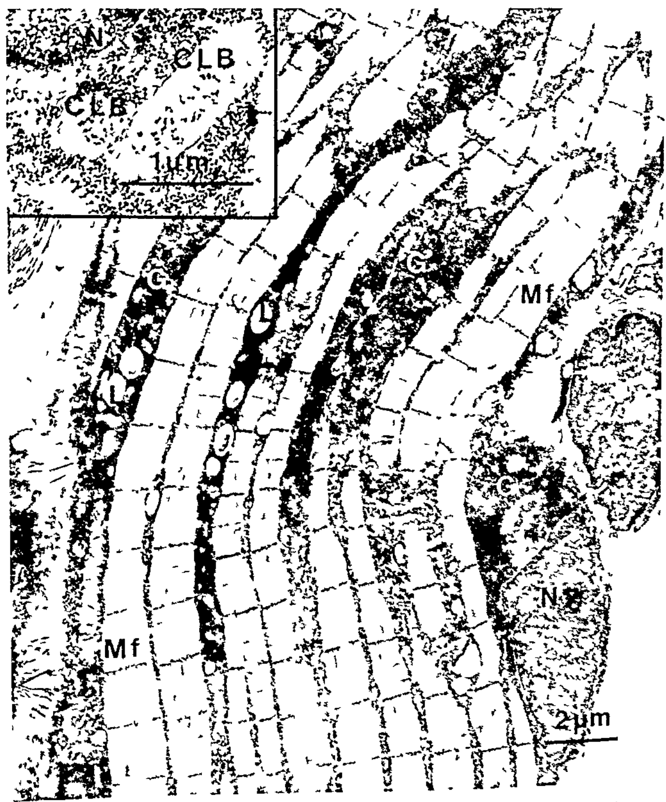


Fig 2 Skeletal muscle cell A high glycogen (*G*) content can be demonstrated between the myofilaments (*Mf*) and near the nucleus (*N*) Lipid droplets (*L*) ×9 000 *Inset* Concentric laminated bodies (*CLB*) Nucleus ×30 000

d facilitate the synthesis of thiaminetri osphate (TTP) Roche & Reed (26) have monstrated the inhibiting effect of thiamine rophosphate on phosphorylation of pyru te dehydrogenase Hence the pyruvate de drogenase is maintained in its active form his activation by thiamine could also explain e decrease of pyruvate and lactate Animal xperiments by Hommes et al (13) support ese findings The relative shortage of ox loacetate due to activation of the pyruvate ehydrogenase complex may be prevented by he administration of aspartate

ACKNOWLEDGEMENT

We are indebted to Dr F A Hommes Department of Pe iatrics University of Groningen The Netherlands for enzymic assay of the liver tissue and to Dr J R Cooper Department of Pharmacology Yale University New Ha ven Connecticut for testing the urine for the inhibitor The technical assistance of Mrs V Greving and Mrs M Herwig is gratefully acknowledged

REFERENCES

1 v Bassewitz D B Roessner A Nauta R & Themann H Cytoplasmic fusions between liver parenchymal cells and infiltrating cells in chronic ag gressive hepatitis *Virchows Arch [Path Anat]* 359 309 1973

Clayton B E Dobbs R H & Patrick A D Leigh s subacute necrotizing encephalopathy Clini cal and biochemical study with special reference to therapy with lipoate *Arch Dis Child 42* 467 1967

Cooper J R Itokawa Y & Pincus J H Thiamine triphosphate deficiency in subacute necrotizing en cephalomyelopathy *Science 164* 74 1969

Cooper J R & Pincus J H Thiamine triphosphate deficiency in Leigh s disease (subacute necrotizing encephalomyelopathy) *In* F A Hommes & C J Van Den Berg (eds) *Inbo n errors of metabolism* Academic Press London & New York 1973 p 119

5 Czok R & Lamprecht W Pyruvat *In* H U Berg meyer (ed) *Methoden der enzymatischen Analyse* Verlag Chemie Weinheim 1970 2 Auflage Band II p 1407

6 Dickinson J C & Hamilton P B The free amino acids of human spinal fluid determined by ion ex change chromatography *J Neurochem 13* 1179 1966

7 Greenhouse A H & Schneck S A Subacute necrotizing encephalomyelopathy A reappraisal of the thiamine deficiency hypothesis *Neurology (Min neap) 18* 1 1968

8 Grover W D Auerbach V H & Patel M S Biochemical studies and therapy in subacute necrotiz ing encephalomyelopathy (Leigh s syndrome) *J Pediatr 81* 39 1972

9 Grundig E Trennungsgang zur quantitativen Bestim mung von Keto- und Aminosauren in kleinen Mengen des Liquor cerebrospinalis *Clin Chim Acta 7* 498 1962

10 Gruskin A B Patel M S Linshaw M Ettenger R Huff D & Grover W Renal function studies and kidney pyruvate carboxylase in subacute necrotizing encephalomyelopathy (Leigh s syn drome) *Pediatr Res 7* 83? 1973

11 Hohorst H J Lactat *In* H U Bergmeyer (ed) *Methoden der enzymatischen Analyse* Verlag Chemie Weinheim 1970 2 Auflage Band II p 1425

12 Hommes F A Polman H A & Reerink J D Leigh s Encephalomyelopathy an inborn error of gluconeogenesis *Arch Dis Child 43* 423 1968

13 Hommes F A Berger R & Luit De Haan G The effect of thiamine treatment on the activity of pyru vate dehydrogenase Relation to the treatment of Leigh s encephalomyelopathy *Pediatr Res 7* 616 1973

14 Jellinger K & Seitelberger F Subacute necrotizing encephalomyelopathy (Leigh) *Ergeb Inn Med Kinderheilkd* 29 155 1970

15 Kamoshita S Aguilar M J & Landing B H Infantile subacute necrotizing encephalomyelopathy *Am J Dis Child 116* 120 1968

16 Landon J Fawcett J K & Wynn V Blood pyru vate concentration measured by a specific method in control subjects *J Clin Pathol 15* 579 1962

17 Laudahn G Fermentaktivitaten und Konzentration von Stoffwechselzwischenprodukten im Blut bei Leber und Herzkrankheiten *Klin Wochenschr 37* 850 1959

18 Leigh D Subacute necrotizing encephalo myelopathy in an infant *J Neurol Neurosurg Psychiatry 14* 216 1951

19 Mair W G P & Tomé F M S *Atlas of the ultrastructure of diseased human muscle* E & S Livingstone Edinburgh & London 1972 p 53

20 Montani S & Perret C Acidose lactique du liquide céphalorachidien dans les meningites bactériennes *Schweiz Med Wochenschr 94* 1552 1964

21 Montpetit V J A Andermann F Carpenter S Fawcett J S Zborowska Sluis D & Giberson H Subacute necrotizing encephalomyelopathy—a re view and a study of two families *Brain 94* 1 1971

22 Mortier W & Michaelis E Die subakute nekrotisierende Enzephalomyelopathie bei eineiigen Zwillingen Pathogenetische und therapeutische Aspekte *Monatsschr Kinderheilkd 121* 294 1973

23 Pincus J H Cooper J R Itokawa Y & Gumbinas M Subactute necrotizing encephalo myelopathy *Arch Neurol 24* 511 1971

24 Pincus J H Subacute necrotizing encephalo myelopathy (Leigh s disease) a consideration of clinical features and etiology *Dev Med Child Neurol 14* 87 197?

25 Pincus J H Cooper J R Murphy J V Rabe

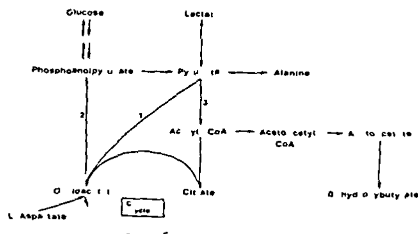


Fig 4 Pyruvate metabolism Main path ways of gluconeogenesis Numbers indi cate *1* pyruvate carboxylase *2* phospho-enolpyruvate carboxykinase *3* pyruvate dehydrogenase complex

At present the question remains unresolved whether a deficiency of pyruvate carboxylase which first was noted by Hommes et al (12) and subsequently confirmed by other authors (29 30 32) is the primary cause of SNE In our patient a negligible amount of pyruvate carboxylase was found in the liver Further evidence for disturbed gluconeogenesis was the lack of an increase in blood glucose after a single dose of alanine However the normal blood glucose even after fasting and espe cially the high amount of glycogen in several tissues are in opposition to a decreased gluconeogenesis Ultrastructural investiga tions of liver specimens of the two children reported by Mortier & Michaelis (22) were performed by one of us and also revealed an increased glycogen content These mor phological findings suggest either increased gluconeogenesis or impaired glycogenolysis in SNE

Grover et al (8) reported a normal activity of pyruvate carboxylase in a child at the age of 10 months but they found a reduced activity in the liver and in kidney tissue at the time of death These findings would support the hypothesis that the decreased pyruvate carboxylase activity is secondary and may de velop in the course of the disease

Our morphological findings confirm earlier light microscope observations Jellinger & Seitelberger (14) and Kamoshita et al (15) de scribed a high glycogen content and a fatty transformation of the liver parenchyma In skeletal muscle cells an increased amount of glycogen was also reported by Jellinger & Seitelberger (14)

The significance and exact nature of the concentric laminated bodies observed in the skeletal muscle of our patient remain un known These unusual structures have been found in several conditions (19)

Pincus et al have advocated treatment of SNE with thiamine or thiamine derivatives (23–25) In a recent report (25) six of 12 pa tients so treated showed signs of remission lasting from 3 months to 2 years In addition the clinical improvement a significant d crease of pyruvate and lactate concentratio in the blood was noted The beneficial effec of thiamine treatment have subsequently bee confirmed by other authors (13 22) In ou patient despite decreased blood pyruvate an lactate levels there was no evidence of an clinical improvement As we started th treatment at the age of 18 months the CN functions might already have been irreversibl impaired Grover et al (8) and Gruskin et al (10) reported therapeutic trials with thiamin and lipoic acid in the same patient the treat ment having been started at the age of 1 months They did not observe signs of clinica improvement

The reason for the efficacy of thiamine treatment in SNE is not yet well understood According to the hypothesis of Pincus et al (25) high doses of thiamine may overcome the inhibition of TPP ATP phosphotransferase

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VARIATIONS OF PROTEASE INHIBITORS IN FOETUSES NEWBORN INFANTS AND IN SOME NEONATAL DISORDERS

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ABSTRACT Sveger T and Ekelund H (Departments of Clinical Chemistry and of Paediatrics Malmo General Hospital Malmo Sweden) Variations of protease inhibitors in foetuses newborn infants and in some neonatal disorders. Acta Paediatr Scand 64 763 1975 —Low levels of protease inhibitors have been found on the 1st day of life in IRDS infants 19 IRDS infants were studied together with foetuses and control term and preterm infants α_1 antitrypsin antichymotrypsin and α_2-macroglobulin were measured with the electroimmuno assay IRDS infants had significantly reduced concentration of α_1 antitrypsin and antichymotrypsin on the 1st day the level increasing to normal on the 2nd day In foetuses α_1 antitrypsin was normal antichymotrypsin 2% and α_2-macroglobulin 1/3 of the normal adult level The protease inhibitors are increased in infants born after premature rupture of foetal membranes The part if any played by protease inhibitors is not entirely understood The inhibitors may theoretically be of some importance in the dissolution of the hyaline membranes protect against pulmonary vasoconstriction protect pulmonary tissue against leucocyte and macrophage proteolytic enzymes and inhibit the release of or counteract vasoactive substances that might take part in the development of shock in IRDS babies

KEY WORDS Idiopathic respiratory distress syndrome (IRDS) protease inhibitors α_1 antitrypsin antichymotrypsin α_2-macroglobulin foetus

The main protease inhibitors α_1 antitrypsin and α_2 macroglobulin varies during the first days of life in the normal newborn and newborn with hyaline membrane disease The variation of antichymotrypsin in the neonatal period has not been studied before

α_1 Antitrypsin an α_1 glycoprotein and acute phase reactant constitutes the major part of the α_1 globulin band in the serum protein electrophoretic pattern It is an inhibitor of leucocytal elastase (29) collagenase (22) pancreatic chymotrypsin and trypsin (2) Emphysema (5) and cirrhosis (1 27) have been reported in connection with inherited variants of α_1 antitrypsin deficiency Low levels have been found during the first day of life in premature infants with hyaline membrane disease (4 6 7 20) α_1 Antitrypsin has been demonstrated in hyaline membranes (20) Kotas et al (17) thought that a low level might be used for detecting those infants with hyaline membrane disease probably requiring special neonatal care

Antichymotrypsin an α_1 globulin is an acute phase reactant and an in vitro protease inhibitor It was first described by Heimberger & Haupt (14) Its molecular size is similar to that of α_1 antitrypsin Its name is misleading since it does not act as a primary chymotrypsin inhibitor in vivo (24) It is a sensitive acute phase protein but its biologic function is not known

α_2-Macroglobulin the main protein of the α_2 fraction in the serum electrophoretic pattern is a polyvalent inhibitor of some proteolytic enzymes (9 10 24) During in

E F Lonsdale D & *Dunn H G* *Thiamine* derivatives in subacute necrotizing encephalomyelopathy A preliminary report *Pediatrics 51* 716 1973

26 Roche T E & Reed L J Function of the nonidentical subunits of mammalian pyruvate dehydrogenase *Biochem Biophys Res Commun 48* 840 1972

27 Sipe J C Leigh s syndrome The adult form of subacute necrotizing encephalomyelopathy with predilection for the brainstem *Neurology (Minneap) 23 1030 1973*

28 Spackman D H Stein, W H & Moore S Automatic recording apparatus for the use in the chromatography of amino acids *Anal Chem 30* 1190 1958

29 Tada K *Yoshida T Konno T Wada Y* Yokoyama Y & Arakawa T Hyperalaninemia with pyruvicemia *Tohoku J Exp Med 97* 99 1969

30 Tang T T Good T A Dyken P R Johnson S D McCready S R Sy S T Lardy H A & Rudolph F B Pathogenesis of Leigh s encephalomyelopathy *J Pediatr 81* 189 1972

31 Utter M F & Keech D B Pyruvate carboxylase I Nature of reaction *J Biol Chem 238* 2603 1963

32 Yoshida T Tada K Konno T & Arakawa T Hyperalaninaemia with pyruvicaemia due to pyruvate carboxylase deficiency of the liver *Tohoku J Exp Med* 99 121 1969

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Table 2 *The concentration mean and standard deviation of α_1 antitrypsin antichymotrypsin and α_2-macroglobulin and albumin on the 1st and 2 nd day of life in the different groups (when no value is given the parameter was not recorded)*

Abbreviations in tables and figures α_1 antitrypsin=α_1 AT α_2-macroglobulin=α_2-M antichymotrypsin=Ach albumin=Alb

Group of infants Range of gestational age	α AT (g/l)	Ach (%)	α_2-M (g/l)	Alb (g/l)
Day 1				
Term (39–42 wks)	1 66 (0 30)	67 (8)	2 92 (0 38)	34 2 (2 8)
Foetus (14–24 wks)	1 66 (0 56)	2 (1)	0 56 (0 20)	16 2 (4 1)
Preterm c (28–33 wks)	1 69 (0 36)	49 (27)	2 01 (0 48)	28 0 (5 0)
Preterm c (34–38 wks)	1 80 (0 25)	61 (21)	2 46 (0 38)	31 8 (3 9)
IRDS (28–33 wks)	1 14 (0 43)	26 (11)	1 56 (0 53)	25 7 (5 4)
IRDS (34–38 wks)	1 29 (0 47)	28 (9)	1 71 (0 40)	28 0 (3 4)
RDS (31–35 wks)	1 80 (0 39)	71 (55)	1 88 (0 64)	29 3 (4 7)
Preterm + prem rupt of the f m (32–34 wks)	2 68(0 58)	180 (25)	2 50 (0 72)	27 5 (5 7)
Day 2				
Term (39–42 wks)	1 70 (0 32)	107 (40)	–	–
Preterm c (28–33 wks)	1 94 (0 38)	109 (50)	1 99 (0 51)	26 1 (2 6)
Preterm c (34–38 wks)	1 77 (0 39)	93 (26)	1 96 (0 51)	31 1 (2 8)
IRDS (34–38 wks)	2 13 (0 63)	88 (45)	1 48 (0 31)	28 6 (8 9)

From Ganrot (11)

The protein was determined with the electroimmuno assay (18) with rabbit antisera prepared at the laboratory The concentrations of the proteins studied in a blood donor serum pool (1 000 donors mainly men) was regarded as 100% This is equivalent to 2 0 g α_1 antitrypsin/l and 1 8 g α_2-macroglobulin/l No pure antichymotrypsin was available The results are given relative to the serum pool 100% antichymotrypsin corresponding roughly to 30 mg/l (26)

RESULTS

The number of infants in each group gestational age birthweight and number who survived and died is given in Table 1 The determination of α_1 antitrypsin antichymotrypsin α_2 macroglobulin and albumin in the different groups on the 1st and 2nd day of life are given in Table 2 The protease inhibitor/albumin ratios of the preterm and IRDS infants are given in Table 3 The distribution of the individual levels of the protease inhibitors and albumin in the preterm control and IRDS groups 28–33 and 34–38 weeks of gestation 1–7 hours after birth are given in Fig 1 Serial protease inhibitor concentrations in 6 IRDS infants during the first 3 days of life are shown in Fig 2

Term infants

As far as we know antichymotrypsin has never before been measured on the 1st day of life It was 67% of normal with a wide inter individual variation and increased on the 2nd

Table 3 *The protease inhibitor/albumin ratio in preterm control and IRDS infants on the first day of life*

For explanation of abbreviations see Table 2

Group of infants Range of gestational age	α AT g/l×100 / Alb g/l	Ach % / Alb g/l	α_2-M g/l×100 / Alb g/l
Preterm c (28–33 wks)	6 2 (1 5)	1 7 (0 8)	7 2 (1 5)
Preterm c (34–38 wks)	5 7 (0 9)	2 0 (0 8)	8 1 (1 7)
IRDS (28–33 wks)	4 5 (1 4)	1 1 (0 5)	6 2 (2 1)
IRDS (34–38 wks)	4 6 (1 3)	1 0 (0 4)	6 2 (1 5)

Table 1 *Number of infants in each group (N) on the 1st and 2nd day of life mean gestational age mean and range of birthweight and number of infants who survived and died*

Group of infants Range of gestational age	N	Gestation weeks (mean)	Birthweight	N survived	N died
1st day					
Term					
39–42 wks	44	40	3 420 (2 910–4 100)	44	–
Foetuses					
14–24 wks	28	19	–	–	–
Preterm control					
28–33 wks	9	31	1 560 (790–2 040)	9	–
34–38 wks	10	36	2 066 (1 400–2 350)	10	–
IRDS					
28–33 wks	8	30	1 385 (750–2 260)	2	6
34–38 wks	11	36	2 315 (2 060–2 640)	8	3
RDS					
31–35 wks	6	33	1 923 (1 400–2 450)	6	–
Preterm+prem rupt of the f m					
32–34 wks	4	33	1 847 (1 690–2 050)	4	–
2nd day					
Term					
39–42 wks	60	40	3 400 (2 910–4 025)	60	–
Preterm control					
28–33 wks	5	31	1 454 (1 020–1 970)	5	–
34–38 wks	5	36	2 088 (1 400–2 300)	5	–
IRDS					
34–38 wks	4	36	2 352 (2 060–2 640)	4	–

trauterine life the α_2 macroglobulin level rises from about one third of the adult level and at term it is 50% above the normal adult value (3) Low levels have been found in acute fibrinolysis (2) and in hyaline membrane disease (4 16)

This paper concerns the variation of these protease inhibitors in foetuses and newborn infants with neonatal disorders especially IRDS (idiopatic respiratory distress syndrome)

CLINICAL MATERIAL

All infants were classified according to gestational age and birth weight with Swedish standard curves as reference (28) Preterm gestational age less than 39 weeks Term gestational age 39–42 weeks The material is summarized in Table 1

The groups were

Term infants all of whom appeared normal

Foetuses delivered by abdominal hysterotomy from women whose pregnancies were interrupted on social and medical grounds

Preterm infants who apart from non haemolytic hyperbilirubinaemia in 3 appeared normal One member of each of 4 pairs of twins was included

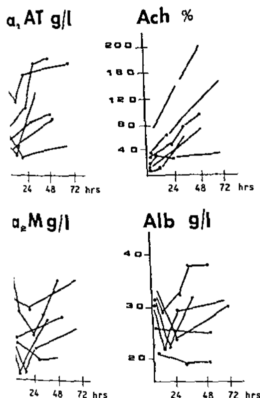
Infants with IRDS (IRDS syn hyaline membrane disease) The condition was diagnosed according to the criteria of Hutchison (15) Infants with coexisting diseases were excluded The infants were treated in an incubator Oxygen was supplied to maintain an adequate arterial oxygen tension fluid (10% glucose 70 ml/kg/day) as well as sodium bicarbonate to correct acidosis given by arterial or venous umbilical catheter None of them were treated with continuous positive airway pressure or respirator Three of those who died belonged to a set of quadruplets All were preterm All IRDS infants who died had hyaline membranes

Infants with RDS (respiratory distress syndrome) consisted of those who did not fill Hutchison s criteria

Preterm infants delivered after premature rupture of the foetal membranes were all apparently healthy

METHODS

Blood samples were obtained by umbilical artery or vein catheter venepuncture or heel puncture The 1st sample from a foetus and term infant was obtained within half an hour and in the other groups 1–7 hous after birth The blood was collected in plastic tubes and plasma or serum was separated and stored at −22 C



2 Serial concentrations of α antitrypsin antichy ...rypsin α macroglobulin and albumin in 6 IRDS in ...s relative to their age in hours after birth For explana... of abbreviations see Table 2

...ants with RDS

...e level of the protease inhibitors and albu...n did not differ from those in the preterm ...ntrol groups The mean and the range of the ...tational ages were not quite comparable

...ants with premature rupture ...the foetal membranes

...ese infants were compared with the preterm ...-38 week old group Both antichymotrypsin ...<0 001) and α_1 antitrypsin ($p<0\ 02$) were ...nificantly increased

...e protease inhibitors and albumin ...the 2nd day of life

...mpared with the 1st day of life the anti...ymotrypsin level for term ($p<0\ 0001$) pre...m control infants ($p<0\ 05$) and those with IRDS ($p<0\ 05$) and the α_1 antitrypsin level was increased in infants with IRDS ($p<0\ 05$) Fig 2 shows that the variation of the proteins during the first 3 days of life in the IRDS infants followed up by serial analyses was considerable

DISCUSSION

The levels and development of some protease inhibitors during intra uterine life are fairly well known (12 25) In our study the concen tration of α_1 antitrypsin in 14–24 week old foetuses was the same as in the term babies Antichymotrypsin has not been determined before There were only traces of it 2% The α_2 macroglobulin concentration was 1/3 of that in normal adults a level in agreement with that found in the previous studies

In infants with IRDS the protease inhibitors α_1 antitrypsin and α_2 macroglobulin are significantly decreased during the 1st day of life (4 6 7 17 20) Our study confirmed most of the previous findings The protease in hibitor/albumin ratio was determined to find out whether the protease inhibitor varied linearly with albumin The protease in hibitor/albumin ratio was significantly de creased in the infants 34–38 weeks of gesta tional age

The sequential analysis of the IRDS infants showed that after the initial low level and further fall in concentration during the first few hours the protease inhibitors and albumin increased rapidly towards normal levels in the surviving infants The low and falling level during the first few hours was probably due to the extravascular leakage of plasma proteins incorporation into hyaline membranes haemodilution and as indicated by the de creased ratio probably at least to some extent to binding to proteases which results in com plex formation elimination and catabolism Since protease inhibitors react as acute phase proteins the stimulation of production can probably explain the rapid rise to normal levels on the 2nd day in the surviving infants

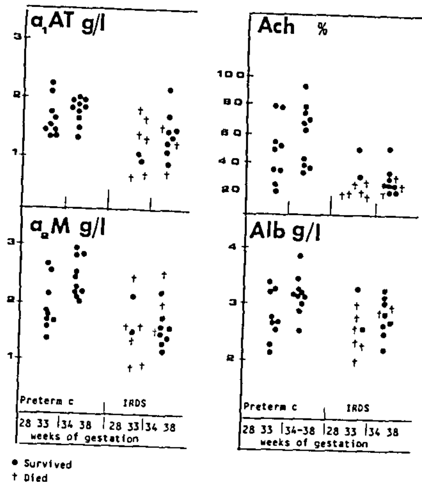


Fig 1 The distribution of the individual levels of α_1 antitrypsin antichymotrypsin α_2 macroglobulin and albumin in preterm control and IRDS infants 28–33 and 34–38 weeks of gestation 1–7 hours after birth For explanation of abbreviations see Table 2

day to a mean of 107% The α_2 macroglobulin and albumin values were taken from a previous study at our laboratory (11) The α_2 antitrypsin level was the same as in that study

Foetuses

The level of antichymotrypsin was only 2% of normal The α_1 antitrypsin level was the same as in term infants The α_2 macroglobulin was about 30% of the normal adult concentration and the albumin was half of the normal concentration of term infants

Preterm control infants

These infants were separated into two groups according to gestational age The concentration of α_2 macroglobulin was significantly ($p<0.05$) lower in the infants of 28–33 weeks of gestational age than in those of 34–38 weeks The level of α_2 macroglobulin in the 28–33 week old infants was significantly lower ($p<0.001$) than in term infants as was the albumin level ($p<0.01$) In the 34–38 week old group only α_2 macroglobulin was significantly lower ($p<0.01$) than in term infants Antichymotrypsin and α_1 antitrypsin did not show any significant variation

Infants with IRDS

The infants with IRDS were divided into two groups with a gestational age of 28–33 and 34–38 weeks and each was compared with the corresponding preterm control groups IRDS infants 28–33 weeks of gestation α_1 antitrypsin ($p<0.02$) and antichymotrypsin ($p<0.05$) were significantly lower The α_1 antitrypsin/albumin ratio was decreased ($p<0.05$) IRDS infants 34–38 weeks of gestation α_1 antitrypsin ($p<0.01$) antichymotrypsin ($p<0.01$) and α_1 macroglobulin ($p<0.001$) were decreased The ratios α_1 antitrypsin antichymotrypsin and α_2 macroglobulin/albumin ($p<0.05$ $p<0.01$ and $p<0.01$ resp were significantly lower

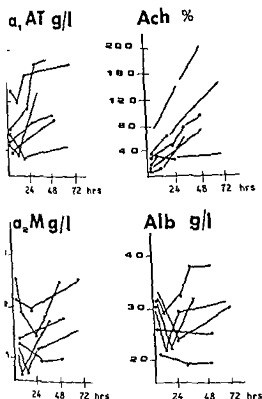


Fig 2 Serial concentrations of α antitrypsin antichymotrypsin α macroglobulin and albumin in 6 IRDS infants relative to their age in hours after birth For explanation of abbreviations see Table 2

Infants with RDS

The level of the protease inhibitors and albumin did not differ from those in the preterm control groups The mean and the range of the gestational ages were not quite comparable

Infants with premature rupture of the foetal membranes

These infants were compared with the preterm 34–38 week old group Both antichymotrypsin ($p<0\ 001$) and α_1 antitrypsin ($p<0\ 02$) were significantly increased

The protease inhibitors and albumin on the 2nd day of life

Compared with the 1st day of life the antichymotrypsin level for term ($p<0\ 0001$) preterm control infants ($p<0\ 05$) and those with IRDS ($p<0\ 05$) and the α_1 antitrypsin level was increased in infants with IRDS ($p<0\ 05$) Fig 2 shows that the variation of the proteins during the first 3 days of life in the IRDS infants followed up by serial analyses was considerable

DISCUSSION

The levels and development of some protease inhibitors during intra uterine life are fairly well known (12 25) In our study the concentration of α_1 antitrypsin in 14–24 week-old foetuses was the same as in the term babies Antichymotrypsin has not been determined before There were only traces of it 2% The α_2 macroglobulin concentration was 1/3 of that in normal adults a level in agreement with that found in the previous studies

In infants with IRDS the protease inhibitors α_1 antitrypsin and α_2 macroglobulin are significantly decreased during the 1st day of life (4 6 7 17 20) Our study confirmed most of the previous findings The protease inhibitor/albumin ratio was determined to find out whether the protease inhibitor varied linearly with albumin The protease inhibitor/albumin ratio was significantly decreased in the infants 34–38 weeks of gestational age

The sequential analysis of the IRDS infants showed that after the initial low level and further fall in concentration during the first few hours the protease inhibitors and albumin increased rapidly towards normal levels in the surviving infants The low and falling level during the first few hours was probably due to the extravascular leakage of plasma proteins incorporation into hyaline membranes haemodilution and as indicated by the decreased ratio probably at least to some extent to binding to proteases which results in complex formation elimination and catabolism Since protease inhibitors react as acute phase proteins the stimulation of production can probably explain the rapid rise to normal levels on the 2nd day in the surviving infants

The antichymotrypsin concentration in IRDS infants was rather low probably because of insufficient stimulation and production at birth in these infants, compared with asymptomatic preterm babies As mentioned in IRDS infants followed up for 2–3 consecutive days antichymotrypsin rose rapidly to normal or still higher levels

The part played by the protease inhibitors in the pathogenesis of IRDS is not properly understood Insufficient synthesis of surface active pulmonary lecithin probably causes progressive expiratory atelectasis leading to hypoxia, capillary damage and exudation and acidosis that diminishes the pulmonary blood flow (13) The hyaline membranes found within 6–8 hours consist of an accumulation of cell detritus red blood cells and plasma proteins including α_1 antitrypsin (19 20 23) Fribrinolysis may be one mechanism of membrane removal The presence of macrophages and leucocytes in the reparative stage indicates participation of leukoproteases in the dissolution of the membrane The α_1 antitrypsin in the hyaline membrane may theoretically interact with and thereby retard the enzymatic activity of the proteases On the other hand α_1 antitrypsin may modify the effect of the proteases and preserve the epithelial lining of the bronchioles the alveoli and the pulmonary capillaries (8)

As mentioned above vasoconstriction of the pulmonary vessels is regarded as one of the components of IRDS In experiments on animals a bovine protease inhibitor Trasylol has proved to block the release of serotonin and histamine and to prevent vasoconstriction (30) Severe IRDS is connected with different stages of infantile shock There is evidence that tissue injury leads to the activation of proteolytic mechanisms with the release of proteases and vasoactive substances as a result (30) Experiments on animals suggest that protease inhibitors offer protection against the action of vasoactive compounds formed in the degradation of tissue and blood products (30) The protease binding capacity may be decreased in some IRDS infants during the first day of life If so it might increase the risk of harmful effect of the vasoactive substances as well as of the proteases It is however not known whether human protease inhibitors are of relevant significance

The risk of hyaline membrane disease is substantially less in prematures with premature rupture of the foetal membranes (31) Our study confirms the earlier observation (17) that these infants have high levels of protease inhibitors

It has been suggested that the α_1 antitrypsin level during the first few hours of life may be a screening factor for assessing the risk of the hyaline membrane disease In our study the overlapping of values was substantial The concentration of antichymotrypsin in infants with IRDS was very low but the degree of overlapping compared with that in preterm control infants makes also antichymotrypsin unsuitable for predicting the development and outcome of IRDS Antichymotrypsin is a very sensitive acute phase protein but the wide normal variation during the first 3 days makes it unsuitable as a reliable screen for subclinical infections in preterm and IRDS infants during their first week of life

REFERENCES

1 Berg N O & Eriksson S Liver disease in adults with α_1 antitrypsin deficiency N Engl J Med 287 1264 1972
2 Bundy H & Mehl J Trypsin inhibitors of human serum II Isolation of the α_1 inhibitor and its partial characterization J Biol Chem 234 1124 1959
3 Ekelund H Hedner M & Nilsson I M Fibrinolysis in newborns Acta Paediatr Scand 49 33 1970
4 El Bardeesy M W & Johnson M A Serum proteinase inhibitors in infants with hyaline membrane disease J Pediatr 81 579 1972
5 Eriksson S Studies in α_1 antitrypsin deficiency Acta Med Scand Suppl 432 1 1965
6 Evans H E Levi M & Mandl I Serum enzyme inhibitor concentrations in the respiratory distress syndrome Am Rev Respir Dis 101 359 1970
7 Evans H E Keller S & Mandl I Serum trypsin inhibitor capacity and RDS J Pediatr 81 588 1972
8 Evans H E Mandl I & Keller S Respiratory distress syndrome serum enzyme inhibitor levels and

lung tissue elastin composition In Mittman (ed) *Pulmonary emphysema and proteolysis* New York Academic Press New York 1972 pp 91–98
9 Ganrot P O Inhibitor of plasmin activity by α_2 macroglobulin *Clin Chim Acta 16* 328 1967
0 — Studies on serum protease inhibitors with special reference to α_2 macroglobulin *Acta Univ Lund II* 7 1967
11 — Variation of the concentrations of some plasma proteins in normal adults pregnant women and newborns *Scand J Clin Lab Invest* 29 Suppl 124 83 1971
12 Gitlin D & Biasucci A Development of γG γA γM β1β1A C1 esterase inhibitor ceruloplasmin transferrin hemopexin haptoglobin fibrinogen plasminogen α antitrypsin orosomucoid β lipoprotein α_2 macroglobulin and prealbumin in the human conceptus *J Clin Invest 48* 1433 1969
13 Gluck L & Kulovich M Fetal lung development *Pediatr Clin North Am* 20 367 1973
14 Heimberger H & Haupt H Karakterisierung von α χ glycoprotein als Chymotrypsin Inhibitor des Humanplasmas *Clin Chim Acta 12 116* 1965
15 Hutchison J J Kerr M M Douglas T A Inall J A & Crosbie J C A therapeutic approach in 100 cases of respiratory distress syndrome of the newborn infant *Pediatrics 33* 956 1964
16 Hyvarinan M A Graven S N & Stiehm E R Development of fibrinolytic proteins in health and disease *Pediatr Res 4* 4/0 1970
17 Kotas R V Fazen I E & Moore T E Umbilical cord serum trypsin inhibitor capacity and the idiopathic respiratory distress syndrome *J Pediatr 81* 593 1972
18 Laurell C B Electro immuno assay *Scand J Clin Lab Invest 29* Suppl *124* 21 1972
19 Lauweryns J M Hyaline membrane disease in newborn infants Macroscopic radiographic and light and electron microscopic studies *Human Pathol 1* 175 1970
0 Mathis R K Freier F F Hunt C E Krivit W & Sharp H L α antitrypsin in the respiratory distress syndrome *N Engl J Med* 288 59 1973
1 Ohlsson K Interaction between bovine α chymotryp in and the protease inhib tors of human macroglobulin in hemostatic balance *Nature 239* 5 1971
2 Pierce J Eiser A & Dhinga H K Pathogenesis of emphysema in antitryps n deficiency *Clin Res 17* 477 1969
3 Potter E L Pulmonary pathology in the newborn *Ad Pediat VI* 157 1953
4 Rinderknecht H & Geokas M C Role for α_2-macroglobulin in hemostatic balance *Nature* 239 116 197
5 Scheidegger J J Martin E & RioHow G L apparation des diverses composantes antigénétiques du sérum au cours du développement foetal *Schwei Med Wschr 86* 224 1956
6 Schwick H G Heimberger N & Haupt H Antiproteinasen des Humanserums *Z Ges Inn Med 21* 193 1966
27 Sharp H L Krivit W & Bridges R A Cirrhosis associated with α_1 antitrypsin deficiency a previously unrecognized inherited disorder *J Lab Clin Med 73* 934 1969
28 Sterky G Swedish standard curves for intrauterine growth *Pediatrics 46* 7 1970
29 Turino G M Senior R M Gary B D Keller S Levi M M & Mandl I Serum elastase inhibitor deficiency and α antitrypsin deficiency in patients with obstructive emphysema *Science 165* 709 1969
30 Weiner R & Zweifach B Vasoinhibitory action of proteolytic enzyme in the capillary bed *Am J Physiol 211* 725 1966
31 Yoon J J & Harper R B Duration of ruptured membranes and risk of respiratory distress *Pediatrics 52* 161 1973

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ADDENDUM

Protease inhibitors have also been estimated in premature infants whose mothers had been treated with betamethasone more than 1 day antepartum in an attempt to prevent hyaline membrane disease Cortisone is a wellknown inducer not only of surfactant but also of intestinal and liver enzymes The number of infants was 9 mean gestational age 35 (range 31–38) weeks and mean birth weight 2310 (1220–3000) g

The concentration mean and standard deviation on the 1st day of life of α_1 antitrypsin was 2 32 (0 72) g/l antichymotrypsin 63 (38)% and α_2 macroglobulin 3 56 (0 50) g/l This group is not quite comparable with the preterm control group but α_2 macroglobulin seems to be increased

LETTER TO THE EDITOR

Sir,

In a recent issue of your journal Dr Rask-Madsen et al (4) quoted our original work (2) measuring potential difference between rectal mucosa and perianal skin using a reference electrode placed on the forearm Using this indirect technique we demonstrated an altered potential difference in cystic children compared with control adults and normal children At this time we suggested this might be due to an alteration in the rectal mucosal potential or altered skin potential, or both in cystic fibrosis

Further work (3) was not noted by Dr Rask Madsen et al We measured skin and rectal charges directly by connecting our reference electrode to the blood stream through an intravenous line Using this technique we showed that the cystic fibrosis patients (C F P) have an abnormal perianal skin potential difference compared with readings we obtained in normal children

C F P mean skin potential difference −40 mV (range −22 to −50 mV)
Control mean skin potential difference −12 mV (range −5 to −20 mV)

There was little difference in the transmural rectal potential difference between our cystic patients and previously reported values in normal adults (1)

C F P mean rectal potential difference −24 mV range ±5 mV (I S D)
Control adults mean rectal P D −33 mV ±6 mV (I S D)

These figures do explain the decreased or reversed rectal potential difference we found using the indirect method

Thus the recent paper (4) confirms our findings (3) of normal polarity of the rectal mucosal surface and the increased skin polarisation in cystic fibrosis

We also made further indirect measurements of skin potential by comparing forearm and finger tip potential difference in cystic and control children In 13 cystic patients the mean forearm–finger tip P D was −42 mV whilst in 13 control children the P D was −16 mV We did not continue with this study as external conditions (room and skin temperature presence of sweating etc) were difficult to standardise but feel it may be worth further investigation

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REFERENCES

1 Archampong E Q & Edmonds C J Effects of luminal ions on the transepithelial electrical potential difference of human rectum *Gut 13* 559 1972
2 Darby C W Measurement of electrical potential difference across colonic mucosa in cystic fibrosis patients *First Annual Meeting European Working Group for Cystic Fibrosis* Stockholm 1970 (abstract)
3 Darby C W & Edmonds C J Electrical potential difference of rectal mucosa and skin in cystic fibrosis patients and normal subjects *Cystic Fibrosis Research Workers Conference* Manchester 1970 (abstract)
4 Rask Madsen J Schiøtz P O Bartels U Nielsen M D & Becher Christensen I Electrical polarisation of rectal mucosa and excretion of tetrahydroaldosterone in patients with cystic fibrosis of the pancreas and in normal subjects *Acta Paediatr Scand 64* 81 1975

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SHORT COMMUNICATION

ELEVATION OF DIPHENYLHYDANTOIN AND PRIMIDONE SERUM CONCENTRATION BY ADDITION OF DIPROPYLACETATE A NEW ANTICONVULSANT DRUG

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In the therapy of convulsive disorders the combination of drugs is necessary particularly frequently Dipropylacetate (DPA) is a sub stance only recently introduced into paediatric therapy for use in the treatment of petit mal absences It presumably raises the concentra tion of gamma aminobutyric acid in the brain Several authors (1 2) have described the potentiating effect of DPA on barbiturate therapy often necessitating a dose reduction For a period of two years we have in the course of treating children made routine serum determinations of various antiepileptic drugs (diphenylhydantoin primidone pheno barbital and nitrazepam) in our Clinical Phar macological Laboratory Fourteen children took a combination of DPA with diphenyl hydantoin or primidone and in 9 of 14 cases the serum concentrations of these latter substances could be measured before and after DPA therapy was begun After only a few days considerable *rises in diphenylhydantoin or* primidone concentrations could be seen although dosage remained constant After considerable reduction in dosage of diphenyl hydantoin or primidone normal serum levels could be achieved (Table I) During further therapy however the dosage of diphenyl hydantoin or primidone had to be increased to the previous levels in some of the children because the serum concentrations had fallen again in spite of continuation of DPA therapy This effect was seen in all patients except one

Two of the 9 children who had low primidone concentrations although taking DPA were first examined after several weeks

Table 1 *Serumconcentrations of antiepileptic drugs during simultaneous therapy with dipropylacetate*

In parentheses daily oral dose of diphenylhydantoin

Pats	DPH-conc before and after onset of DPA therapy[a] Before	After	DPH-conc 1–3 mo after onset of DPA therapy
1	9 µg/ml (8 mg/kg)	?2 µg/ml (8 mg/kg)	–
2	5 µg/ml (7 mg/kg)	15 µg/ml (7 mg/kg)	8 µg/ml (7 mg/kg)
3	1? µg/ml (9 mg/kg)	5 µg/ml (9 mg/kg)	14 µg/ml (8 mg/kg)
4	4 µg/ml (6 mg/kg)	15 µg/ml (6 mg/kg)	6 µg/ml (6 mg/kg)
5	12 µg/ml (10 mg/kg)	31 µg/ml (10 mg/kg)	9 µg/ml (8 mg/kg)

DPH = Diphenylhydantoin
[a] DPA = Dipropylacetate

Table 2 *Serumconcentrations of antiepileptic drugs during simultaneous therapy with dipropylacetate*

In parentheses daily oral dose of Primidone

Pats	Primidone-concentrations before and after onset of DPA therapy[a]		Primidone conc 1–3 mo after onset of DPA therapy
	Before	After	
1	4 μg/ml (10 mg/kg)	14 μg/ml (10 mg/kg)	–
2	6 μg/ml (15 mg/kg)	12 μg/ml (15 mg/kg)	5 μg/ml (14 mg/kg)
3	8 μg/ml (18 mg/kg)	13 μg/ml (18 mg/kg)	14 μg/ml (16 mg/kg)
4	5 μg/ml (15 mg/kg)	12 μg/ml (15 mg/kg)	8 μg/ml (15 mg/kg)
5	8 μg/ml (15 mg/kg)	21 μg/ml (15 mg/kg)	–
6	10 μg/ml (17 mg/kg)	25 μg/ml (17 mg/kg)	7 μg/ml (16 mg/kg)
7	6 μg/ml (14 mg/kg)	14 μg/ml (14 mg/kg)	–
8	–	–	4 μg/ml (16 mg/kg)
9	–	–	2 μg/ml (20 mg/kg)

DPA = Dipropylacetate

of DPA therapy We believe that DPA initially causes a considerable slowing or blocking of diphenylhydantoin and primidone metabolism (presumably of barbiturates and other substances also) After a longer period of application this effect may be lost and the drug may even cause acceleration of metabolism of the substances mentioned This does not seem to apply to all individuals however

In addition to coagulation defects described by Sutor et al (3) occurring during DPA therapy this mechanism seems to be worthy of consideration

In summary this report is given in order to stress once again the importance of routine serum concentration determinations of anticonvulsant drugs during therapy

REFERENCES

1 Neuhauser G & Aebert I Epilepsie Behandlungsgrundsatze und spezielle Therapie mit Ergenyl® *Kolloquium* Munchen 1973
2 Volzke E & Doose H Dipropylacetate (Dekapine® Ergenyl®) in the treatment of epilepsy *Epilepsia (Amst) 14* 185 1973
3 Sutor A & Jesdinsky Buscher Gerinnungsveranderungen durch Dipropylessigsaure (Ergenyl®) *Med Welt 25* 447 1974

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CASE REPORT

SYSTEMIC ALLERGIC REACTION TO INITIAL INSULIN THERAPY IN A JUVENILE DIABETIC

Enhanced Blast Transformation to Insulin

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ABSTRACT Dacou Voutetakis C Thomaidis Th Roma E. Kalpini Mavrou A and Economou Mavrou C (First Department of Paediatrics Athens University Greece) Systemic allergic reaction to initial insulin therapy in a juvenile diabetic Enhanced blast transformation to insulin Acta Paediatr Scand 64 773 1975 —A 14 year-old girl with recent onset of diabetes developed a severe systemic allergic reaction to beef–pork insulin on the third day of insulin therapy The same reaction developed following the injection of pork insulin and monocomponent insulin The patient was induced to tolerate pork insulin by systemic desensitization to this insulin over an interval of 6 days Peripheral blood lymphocytes from the patient cultured in the presence of insulin responded by increased blast cell transformation as compared with controls who either had previously presented local allergic reactions to insulin or never had manifested insulin allergy

KEY WORDS Insulin allergy desensitization blast cell transformation

Severe systemic allergic reactions to insulin impeding insulin therapy are extremely rare in diabetics below the age of 15 years Such reactions develop in adults usually a few days after reinstitution of insulin therapy (5 6)

This communication discusses severe systemic allergy to insulin within 3 days of the initiation of insulin therapy in an adolescent diabetic girl who was successfully desensitized It also reports preliminary observations on blast transformation of lymphocytes in systemic insulin allergy

CASE REPORT

D K a 14 year-old girl had recently lost weight and had developed polyuria and polydipsia one week before admission There was a history of diabetes mellitus in her maternal grandfather his brother and a son of the latter The patient had never had any allergic reaction to drugs or other substances

On admission to the hospital she was moderately dehydrated and had glucosuria and ketonuria (4+) Blood glucose was 360 mg/100 ml and carbon dioxide content was 11 mEq/l Treatment with crystalline beef pork insulin was started immediately

On the third day of treatment 10 min after the 9th injection of insulin she complained of generalized severe itching and a burning sensation at the same time she developed an intense erythema on her face neck and anterior chest. A fine vesicular rash appeared on her neck and upper chest All symptoms and signs subsided about 0 minutes following the intravenous injection of an antihistaminic (Sandostene Calcium 10 ml Thenaldine 50 mg and calcium glycolactobionate 1 375 g) except for the tiny vesicles which were retained for 3 days

The patient developed a similar but severe and more prolonged reaction after another injection of crystalline pork insulin She was then given desensitizing doses of monocomponent insulin (1 2) (MC) i e 0 1 unit was first administered subcutaneously then gradually the dose was increased at 10-minute intervals until 10 units were given after 1 1/2 hour Three hours following the last injection of

Table 1 *Schedule for desensitization to crystalline pork insulin*

Day	Hour	Insulin dose (units)	Route of administration
1	19	0 00001	Intradermal
	23	0 0001	Intradermal
2	8 30	0 001	Intradermal
	12	0 01	Intradermal
	16	0 1	Intradermal
	20	1	Intradermal
3	8	2	Subcutaneous
	12	4	Subcutaneous
	16	6	Subcutaneous
4	11	8	Subcutaneous
	14	10	Subcutaneous
	18	10	Subcutaneous
5	8	15	Subcutaneous
6	8	20	Subcutaneous

MC insulin she again complained of generalized itching and burning sensation and a diffuse erythema was noted on her face neck chest and extremities Her symptoms were less intense than with previous insulin preparations Subsequently another slower desensitization schedule was tried (3) Commercially available crystalline pork insulin was administered initially in a dose of 0 0001 U by the intradermal route the dose was gradually increased to 1 unit after 2 days and to 10 units after 4 days (Table 1) (7) This procedure did not cause any systemic or local reaction at the site of injection

During the various therapeutic trials the patient was maintained in satisfactory biochemical equilibrium by intravenous fluid and electrolyte therapy and a strict diet Tolbutamide 1500 mg daily was also given in an attemp to mobilize any insulin reserve Adrenaline hydrocortisone and a tracheostomy tray were available at the patient s bedside However angioneurotic edema neve occurred

A change to intermediate acting pork insulin (isophane NPH) did not cause any reaction During the next 12 months control of diabetes was maintained with 10 units of crystalline and 25 units of isophane NPH pork insulin with no allergic reaction

Immunological studies Serum IgG IgM and IgA (determined by radial immunodiffusion) were 800 mg/100 ml 200 mg/100 ml and 110 mg/100 ml respectively These values are normal for the Greek population of this age (8) Peripheral blood lymphocytes from the patient 7 diabetics on insulin therapy and 3 healthy controls were cultured in TC 199 medium for 7 days in the presence of different insulin preparations at two concentrations (0 004 and 0 008 u/ml of medium respectively) In each case the insulin added to the culture was that used by the patient in some controls the insulin used by the index case was also tested The patient s lymphocytes were cultured twice 25 days and 12 months following initiation of insulin therapy The results of the lymphocyte stimulation tests and the types of insulin used are given in Table 2 The percentage of transformed cells following stimulation with insulin was repeatedly much higher in the patient than in three diabetic children who received insulin for one two and four years respectively and had developed local reactions at the sites of injection It was also considerably higher than in three diabetic children who had received insulin for only 7 to 20 days and had not developed any allergic reaction (Table 2) Twelve months later blast transformation of the patient s lymphocytes in response to various types of insulin was still enhanced but to a lesser degree when compared to that 25 days after the systemic allergic reaction (Table 3)

Table 2 *Blast transformation of cultured peripheral blood lymphocytes in each subject*

Subject	Age (years)	Duration of insulin therapy	Allergic reaction to insulin	% transformed blast cells								
					Crystalline		Actrapid[b]		Lente		Semilente[a]	
				PHA	A[d]	B	A	B	A	B	A	B
Index case DK	14	25 days	Systemic	82	37	57	37	49				
Index case DK	15	12 months	Systemic	72	28	14	13	26				
Diabetics												
N H	12	20 days	None	85		10	14					
V D	13	7 days	None	83	4	4		4				
M A	5	9 days	None	85	9	15	10	5				
D E	11	1 year	Local	84							10	8
M D	9	2 years	Local	80					11	10		11
G A	11	4 years	None	73							6	4
G M	8	4 years	None	81		9		9				
Healthy												
K H	45	None		76	3	3						
M D	13	None		84	6	7						
T B	11	None		80	3	1		2				

[a] Beef pork insulin [b] Pure pork insulin [c] Phytothemagglutinin [d] Insulin in medium 0 004/ml Insulin in medium 0 008/ml

Table 3 *Blast transformation of cultured peripheral blood lymphocytes*

Mean and range in each group

Subjects	*n*	No of experiments	% transformed blast cells	
			Mean	Range
Index case				
25 days	1	4	35	37–57
12 months	1	4	0 5	13–28
Diabetic controls	7	18	8 5	4–15
Healthy controls	3	7	3 5	1–7

DISCUSSION

The development of a generalized allergic reaction to insulin in a 14-year-old diabetic girl only 3 days following the first injection of insulin is exceedingly rare and remains unexplained Both local and the much rarer (7) generalized manifestations of insulin allergy usually occur when insulin therapy is reinstituted following variable periods without insulin (5 6) It is difficult to explain why the intradermal injection of insulin caused no wheal and erythema (3) in our patient

Generally allergy to insulin has been attributed not only to the insulin molecule but also to impurities (7) and proteins such as protamine and globin which are added to insulin preparation (5) In our patient systemic allergic reaction developed not only to the usual insulin preparations but also to MC insulin Hence it may be assumed that the allergic reaction was directed against the insulin molecule itself rather than to impurities The more delayed and less intense reaction to MC insulin could be explained by the fact that MC insulin was given in desensitizing doses

Systemic allergic reactions to insulin in adult diabetics can usually be avoided by switching to an effective oral hypoglycemic agent (7) Since these agents are not effective in juvenile diabetes a change in the type of insulin is required and if all types prove allergenic desensitization to insulin should be attempted Our patient reacted unfavorably to all tested types of insulin Densensitization to crystalline pork insulin was then accomplished in 6 days and it was maintained during the subsequent 12 months at follow up

It has been established that reaginic antibodies against insulin develop in systemic reactions to insulin These are IgE antibodies (9) whereas antibodies responsible for insulin resistance are mainly IgG (9 10) Serum IgE and antibodies to insulin were not determined in our patient for technical reasons

Although the humoral response to insulin has been studied extensively little attention has been paid to the delayed hypersensitivity response to insulin in either animals or man It has been shown that insulin produces a state of delayed hypersensitivity in guinea pigs and that this can be elicited with either the intact insulin molecule or with isolated b chain but not with a chain (4) Peripheral blood lymphocytes from diabetic persons have recently been shown to respond by blast cell transformation when cultured with phytohemagglutinin or *Candida albicans* antigen just as do lymphocytes from normal individuals (1) however as far as we know lymphocytes from diabetic patients on insulin therapy have not been challenged with insulin in cultures The observation made in this study that the lymphocyte response to insulin was stronger in the diabetic patient who developed systemic insulin allergy than in patients with either local reactions or no reaction to insulin or in healthy individuals implies that cellular immunity may also be involved in generalized insulin allergy It is hoped that this observation will be confirmed by others It is known that most allergic reactions are not pure and that the blast transformation test has been found to be positive in systemic allergic reactions to a number of drugs (2 11) Hence this test may prove useful in diagnosing insulin allergy Whether it may help to predict dangerous systemic reactions against insulin is a question which cannot be answered yet

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During the various therapeutic trials the patient was maintained in satisfactory biochemical equilibrium by intravenous fluid and electrolyte therapy and a strict diet Tolbutamide 1500 mg daily was also given in an attempt to mobilize any insulin reserve Adrenaline hydrocortisone and a tracheostomy tray were available at the patient s bedside However angioneurotic edema never occurred

A change to intermediate acting pork insulin (isophane NPH) did not cause any reaction During the next 12 months control of diabetes was maintained with 10 units of crystalline and 25 units of isophane NPH pork insulin with no allergic reaction

Immunological studies Serum IgG IgM and IgA (determined by radial immunodiffusion) were 800 mg/100 ml 200 mg/100 ml and 110 mg/100 ml respectively These values are normal for the Greek population of this age (8) Peripheral blood lymphocytes from the patient 7 diabetics on insulin therapy and 3 healthy controls were cultured in TC 199 medium for 7 days in the presence of different insulin preparations at two concentrations (0 004 and 0 008 u/ml of medium respectively) In each case the insulin added to the culture was that used by the patient in some controls the insulin used by the index case was also tested The patient s lymphocytes were cultured twice 25 days and 12 months following initiation of insulin therapy The results of the lymphocyte stimulation tests and the types of insulin used are given in Table 2 The percentage of transformed cells following stimulation with insulin was repeatedly much higher in the patient than in three diabetic children who received insulin for one two and four years respectively and had developed local reactions at the sites of injection It was also considerably higher than in three diabetic children who had received insulin for only 7 to 20 days and had not developed any allergic reaction (Table 2) Twelve months later blast transformation of the patient s lymphocytes in response to various types of insulin was still enhanced but to a lesser degree when compared to that 25 days after the systemic allergic reaction (Table 3)

Table 2 *Blast transformation of cultured peripheral blood lymphocytes in each subject*

Subject	Age (years)	Duration of insulin therapy	Allergic reaction to insulin	% transformed blast cells								
					Crystalline[a]		Actrapid[b]		Lente[a]		Semiler	
				I HA	A[d]	B	A	B	A	B	A	B
Index case DK	14	25 days	Systemic	82	37	57	37	49				
Index case DK	15	12 months	Systemic	72	28	14	13	26				
Diabetics												
N H	12	20 days	None	85		10	14					
V D	13	7 days	None	83	4	4		4				
M A	5	9 days	None	85	9	15	10	5				
D E	11	1 year	Local	84							10	8
M D	9	2 years	Local	80					11	10		11
G A	11	4 years	None	73							6	4
G M	8	4 years	None	81		9		9				
Healthy												
K H	45	None		76	3	3						
M D	13	None		84	6	7						
T B	11	None		80	3	1		2				

[a] Beef pork insulin [b] Pure pork insulin [c] Phytothemagglutinin [d] Insulin in medium 0 004/ml Insulin medium 0 008/ml

Acta Pædiatr Scand 64 777–782 1975

CASE REPORT

FAMILIAL QT PROLONGATION AND RISK OF SUDDEN DEATH

CURT FURBERG and HERJE HÖRNELL

Fro n the Departments f Clinical Physiology and Paediat ics Central H spital Bode S eden

ABSTRACT Furberg C and Hornell H (Department of Clinical Physiology and Paediatrics Central Hospital Boden Sweden) Familial QT prolongation and risk of sudden death Acta Paediatr Scand 64 777 1975.—Two sisters with the syndrome of familial QT prolongation in the ECG and syncope are presented A recently suggested mechanism of the syndrome is presented and preventive measures to reduce the risk of sudden death associated with it are proposed

KEY WORDS Familial disorder QT prolongation sudden death

There have been several reports of families with prolonged QT interval in the ECT and many of the affected subjects have died sud denly since the Surdo-cardiac Syndrome was first described in 1957 by Jervell & Lange Nielsen (8) Congenital deafness is considered as one of the characteristics of this syndrome besides prolonged QT interval in the ECG and spells of syncope that might terminate fatally due to ventricular arrhythmia When a similar syndrome but with normal hearing was rec ognized in 1963 in Italy by Romano et al (16) and in Ireland by Ward (17) it was first thought to be a separate entity This assump tion was supported by reported differences in mode of inheritance (4) However Mathews et al (11) recently described a family where some of the affected members had congenital deafness while others had normal hearing They suggested therefore that the auditory and cardiac defects are inherited separately

A recently proposed mechanism of the syndrome and some suggestions for preven tion of sudden death in affected subjects are briefly presented together with case reports of two sisters with prolonged QT interval

CASE REPORT

Case I

The patient a 13 year-old girl had a syncopal episode at school and was admitted to the Central Hospital Boden some hours later She had earlier been healthy and had no history of syncope prior to this episode Nor was there a family history of syncopal spells sudden death or congen ital deafness The syncope that was preceded by slight lightheadedness and nausea occurred when the patient was resting on a bed The patient had not been exposed to physical or acute emotional stress prior to the attack It was accompanied by convulsions and urinary inconti nence

On admission to hospital she had a normal somatic status including neurological status Blood pressure 115/70 mmHg Heart rate 75 per min and regular Routine blood tests including serum electrolytes liver and muscle enzymes and urine analysis were normal X rays of the chest ophthalmoscopy audiogram and electro-encephalogram were normal Resting 12 lead ECG the day after admission (1 9 197?) showed sinus rhythm and marked QT prolongation no arrhythmia (Fig. 1 left) The prolonged QT interval that indicates the electric systole (11) was accompanied by a normal duration of the mechanical systole as indicated from the phono-cardiogram (Ph 6) (Fig 2) Daily ECG recordings during a week after the initial showed varying QT pro-longation with a tendency to a shortening towards the end of that period Digitalis was prescribed initially but was changed after about 2 months to propranolol Her dose is at present 40 mg×3 She has not had any syncopal attack since the first admission to the hospital 19 months ago A resting ECG about 7 months after the first episode (5 4 1973) was within normal limits (Fig 1 right)

REFERENCES

1 Ablesalam H R Hazlitt B & Cowan D H Response of peripheral blood lymphocytes from patients with diabetes mellitus to phytohaemagglutinin and candida albicans *Diabetes 21* 906 1972
2 Alexander S & Forman L Which of the drugs caused the rash? Or the value of the lymphocyte transformation test in eruption caused by nalidixic acid *Br J Derm 64* 429 1971
3 Arkins J A Engbring N H & Lennon E J The incidence of skin reactivity to insulin in diabetic patients *J Allergy 33* 69 1962
4 Clark C & Unnoz Z Delayed hypersensitivity to insulin and its component polypeptide chains *J Immunology 105* 574 1970
5 Goldstein H H Allergy and diabetes *In* A Marble P White R F Bradley & L P Krall (eds) *Joslin s Diabetes Mellitus* Lea & Febiger Philadelphia 1971 p 701
6 Hanauer L & Batson J M Anaphylactic shock following insulin injection Case report and review of the literature *Diabetes 10* 105 1961
7 Lieberman P Pattersson R Metz R & Lucena G Allergic reactions to insulin *JAMA 215* 1106 1971
8 Morphis L Mandalenaki Asfi C Katis K & Matsaniotis N Unpublished data
9 Patterson R Mellies C Y & Roberts M Immunologic reactions against insulin II IgE anti insulin insulin allergy and combined IgE and IgG immunologic insulin resistance *J Immunology 110* 1133 1973
10 Patterson R O Rourke Y Roberts M & Suszko I Immunologic reaction against insulin I IgG anti insulin and insulin resistance *J Immunology 110* 1126 1973
11 Ripps C S Fellner M J & Hirschhorn K Response of lymphocytes to tetracycline *Lancet 2* 951 1965
12 Schlichtukrull J Brange J Christiansen H Hallund O Heding L G & Jorgensen K H Clinical aspects of insulin antigenicity *Diabetes 21* (Suppl 2) 649 1972

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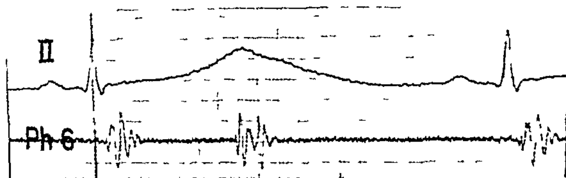


Fig 2 Electrocardiogram lead II in case 1 showing the prolonged electric systole as compared to the mechanical systole obtained from simultaneously recorded phonocardiogram (Ph 6)

Case 2

The patient a 9 year-old girl and only sister to case 1 was diagnosed when she and her parents were called for a screening ECG exam (9 9 1972) Her resting ECG showed sinus rhythm with a QT prolongation (Fig 3) She had never had a syncope Her audiogram was normal as well as routine blood and urine analysis and X rays of the chest The patient was given propranolol and has still not had a syncope 19 months after she was first examined The resting ECG has been normal at 2 check-ups during the last year

The parents had normal ECGs

DISCUSSION

About 100 documented or suspected cases of this syndrome with familial QT prolongation with or without congenital deafness have been described from Europe USA Canada South Africa and Japan (see 9 for references also 2 3 5 7 10–14) Of those cases about two thirds have had normal hearing About one third of all cases have led to sudden death in ventricular fibrillation or asystole mostly in childhood

Screening studies of deaf mutes in several countries have revealed on overall prevalence of the Surdo-cardiac syndrome of approximately 3 5 per 1000 deaf mutes (15) Further cases of the syndrome were found in families

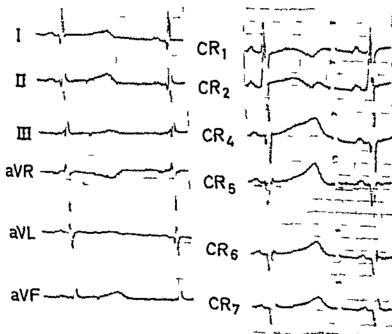


Fig 3 Electrocardiograms in case 2 (Paper speed 50 mm/sec)

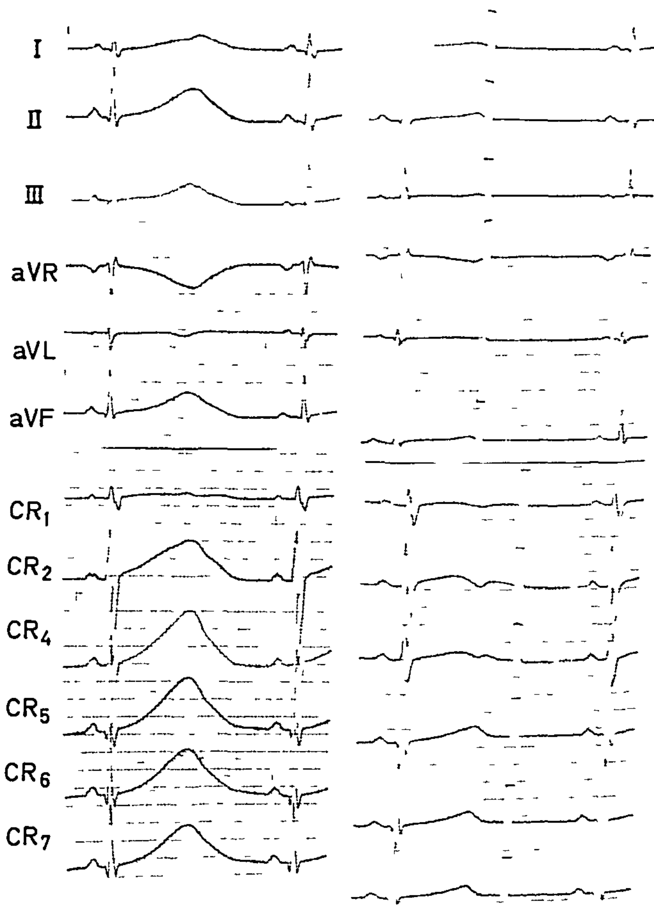


Fig. 1 Electrocardiograms in case 1 the day after admission to hospital (left) and about 7 months later (right) (Paper speed 50 mm/sec)

3 Furlanello F Macca F & Dal Palu C Observation on a case of Jervell and Lange Nielsens syndrome in an adult *Br Heart J 34* 648 1972
4 Gale G E Bosman C K Tucker R B K & Barlow J B Hereditary prolongation of QT interval Study of two families *Br Heart J 3?* 505 19?0
5 Garza L A Vick R L Nora J J & McNamara D G Heritable Q T prolongation without deafness *Circulation 41 39 1970*
6 Han J Garcia de Jalon P & Moe G K Adrenergic effects of ventricular vulnerability *Circ Res 14* 516 1964
7 Hanazono N Yoshihiro A Ohnishi M Oda H Yuhara N Nishio T Ishida H Takeuchi A & Kohashi K Heritable Q-T prolongation without deafness The Romano Ward Syndrome *Jap Heart J 14* 479 1973
8 Jervell A & Lange Nielsen F Congenital deaf mutism functional heart disease with prolongation of the Q-T interval and sudden death *Am Heart J 54* 59 1957
9 Johansson B W & Jorming B Hereditary prolongation of QT interval *Br Heart J 34* 744 197
10 K rhunen P Luomanmaki K Heikkila J & Eisalo A Syncope and Q T prolongation without deafness The Romano-Ward Syndrome *Am Heart J 80* 8 0 19 0
11 Mathews E C Blount A W Jr & Townsend J I Q T prolongation and ventricular arrhythmias with and without deafness in the same family *Am J C di 1* ?9 70? 197?
12 Moss A J & McDonald J Unilateral cervicothoracic sympathetic ganglionectomy for the treatment of long QT interval syndrome *N Engl J Med* ?85 903 1971
13 Olley R M & Fowler R S The surdo-cardiac syndrome and therapeutic observations *Br Heart J 3?* 467 1970
14 Phillips J & Ichinose H Clinical and pathological *studies in the hereditary syndrome of a long QT interval* syncopal spells and sudden death *Chest 58* ?36 1970
15 Prineas R J & Furberg C D Pathogenesis of sudden death due to cardiovascular disease *Cardiologia d Oggi* 1974 (in press)
16 Romano C Gemme G & Pongiglione R Aritmie cardiache rare dell eta pediatrica *Clin Ped atr* (Bologna) *45* 656 1963
17 Ward O C A new familial cardiac syndrome in children *J Ir Med Assoc 54* 103 1964
18 Yanowitz F Preston J B & Abildskov J A Functional distribution of right and left stellate innervation to the ventricles Production of neurogenic electrocardiographic changes by unilateral alteration of sympathetic tone *Circ Res 18* 416 1966

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of affected subjects This prevalence rate is almost certainly an underestimate because the QT prolongation is often intermittent (15) and so some cases would have been missed at a single resting ECG screen Phillips & Ichinose (14) have pointed out that a screen should include a stress test as the ECG may be normal at rest and that the QT prolongation only may show after exercise

When a prolonged QT interval is present refractory periods are much longer in some parts of the myocardium than in other parts Such a disparity is one of the conditions that favors the initiation of ventricular fibrillation through the R on T phenomena A QT prolongation occurs in myocardial necrosis certain stimuli to the autonomic system (6) and after certain drugs such as quinidine (1) Biochemical enzymatic and anatomical mechanisms have been proposed as the underlying cause of familial QT prolongation The most recent theory suggests asymmetrical sympathetic stimulation of the ventricular myocardium as the cause of the syndrome Yanowitz et al (18) demonstrated in dogs that left stellate stimulation produces a prolonged QT interval Recently Moss & McDonald (12) were able to show in a patient with QT prolongation that left stellate ganglionic block normalized the QT interval while a block on the right side caused a further QT prolongation The patient had later a left stellate ganglionectomy performed and the QT interval became normal and here syncopes (with documented ventricular fibrillation) ceased

Although the syndrome with familial QT prolongation is not very common the high rate of sudden death associated with it makes preventive measures important *Firstly* screening for unrecognized cases ought to be done as the first syncopal episode may end fatally The following measures could be undertaken

(*a*) ECG could be recorded more often in children with syncopal spells

(*b*) Continuing programs of ECG screening of deaf children could be undertaken together with screening of families where an affected child is found Repeated ECG recordings a rest or an exercise test should be considere

(*c*) A similar screen ought to be done i families where a child dies suddenly and n cause of the death is found

Secondly prophylactic treatment of recognized cases should be started as soon a possible to prevent syncopal spells as the can end fatally Adrenergic beta blockad (propranolol) has been reported to be the bes method of drug treatment of familial QT prolongation (4 5) However it is not alway effective and is reported not to alter the QT interval (4) The ECG recordings from our tw cases who are treated with propranolol in a dose somewhat higher than that given to previously reported patients show no longer an QT prolongation In our cases it is impossible to determine if this is due to the long term treatment with high doses of propranolol or to spontaneous changes of the QT interval

The action of propranolol is thought to be mediated through its direct antiarrhythmic properties rather than through the beta adrenergic blockade (5) A daily dose of 40–120 mg seems to be effective in treating affected subjects Surviving adults have less frequent syncopal episodes sometimes none at all (4) and treatment should therefore at least be given until the subject reaches adulthood However deaths have been reported to occur in adults (4) The degree of QT prolongation or the frequency and severity of the attacks should be taken into account when the decision is made whether or not the medication should be stopped Surgical treatment with left stellate ganglionectomy might be considered when the response to drug therapy is poor or the clinical history makes it urgent

REFERENCES

1 Bernreiter M Prolonged QT ventricular premature contraction and sudden death *Missouri Med* 67 104 19[illegible]0

2 Bernuth G von Belz G C Evertz W & Stauch M QTU abnormalities sinus bradycardia and Adams Stokes attacks due to ventricular tachyarrhythmia *Acta Paediatr Scand* 62 675 1973

mi roscopy illustrations There is a brief review of the amniotic fluid which is of increasing clinical interest The umbilical cord is presented with very detailed original figures of anatomy and histology The pathogene is of the leucocytic infiltration found in normal cases is discussed and the authors present a new theory related to the rapid slowing of the blood stream through the umbilical vein during delivery

The neonatal electrocardiographic and phonocardiographic findings are presented in relation to gestational age blood volume and pulmonary vascular resistance Normal values of the ECG parameters from the authors own extensive material are given Heart volume variations in the newborn infant are discussed as the method for volume determination The nomograms are provided for calculating the roentgenological heart volume in infants

The fetal development of intimal cushions which are thought to become the site of predilection for lipid infiltration and atherosclerotic plaques have interested the authors very much Thus they believe that the proved lipid changes in the arteries of newborns are the basis for subsequent development of atherosclerosis The pathological changes with calcifications in the arteries luminal layer in segments with a higher hemodynamic load and accelerated growth were found by Meyer It seems however far from proven that these changes are the initial stage of atherosclerosis which is the hypothesis of the authors The used expression borderline pathologic changes sounds more probable It is hard to believe that nature in this very first part of human life starts by developing the most important cause of death in future life

The common occurrence in many arteries in the human as well as in animals and the histological appearance makes it more probable that they are not a pathological finding but a normal anatomical feature as an adaptation to mechanical forces

Numerous figures illustrate and complement the text very well and are generally very illustrative even if the cinéangiograms are of an earlier date and not as clear as technical development now permits The high quality photographs are generous both in size colour and number It is somewhat disturbing however that 8 unnumbered pages of colour figures have been placed in the middle of an early chapter It would have been more convenient if they had been placed in the appendix together with the tables

Every chapter in the book has an extensive list of references which is of importance in the rapidly growing field of information from neonatal research

This volume is not an undergraduate textbook nor is it one for pediatric pathologists the book serves mainly as a reference for neonatologists obstetricians and physicians concerned with care and management of newborn infants as well as for pathologists It will be of greatest value to all those interested in the complexity of the physiological transition of the circulation and the cardiovascular system from fetal to extra-uterine life

The price of the book is low which would suggest that The Foundation for Child Development has subsidized the cost

Claes Thoren

F Schmid (ed) *Padiatrische Radiologie* Band 1 Stutzgewebe Zentralnervensystem Syndrome 580 pp illus DM 248 – Band 2 Thoraxorgane Verdauungstrakt Urogenitaltrakt 525 pp illus DM 248 – Springer Verlag Berlin Heidelberg and New York 1973

These two volumes are the manifestation of an ambitious attempt to create a German text book of paediatric radiology by a single author The first volume is concerned with the connective tissues the central nervous system and the malformation syndromes and the second with the thoracic organs and the digestive and genito-urinary systems Both are amply illustrated with high quality reproductions of radiographs and the chapters on bone radiology include numerous statistical tables on measurements in the growing infant and child

The references are up to date and are a helpful guide to supplementary reading on specific topics This is valuable particularly because unfortunately the text can hardly be considered a reflection of recent progress Since no text book covering so vast a field can keep entirely abreast with the advances in knowledge such criticism may seem unfair but the author perpetuates a number of such inadvertencies and mistakes of the past as ought not reappear in modern literature For example the remarks on congenital dislocation of the hip joint are obsolete and the figure shown as an illustration of mediastinal pleuritis will help to revive rather than avoid the formerly common confusion between this condition and a normal variety of the thymus Differential diagnosis has on the whole received scant attention

Each volume is provided with a meticulous separate index which facilitates consultation They are therefore easily used as a complement to other sources of information but in the opinion of the reviewer they do not fulfil the expectations on a major reference tract in paediatric radiology

Geo g Theander

BOOK REVIEWS

S Godfrey *Exercise testing in children* 168 pp illus W B Saunders Company Ltd London 1974 £5 –

Paediatric exercise physiology is a field which has attracted increasing interest in the past decade This is due to the special problems created in children by growth maturation and the presumed increase in exercise capacity induced by training Nevertheless no real handbook on the exercise physiology of children has been available This book by Simon Godfrey is a good start It considers the special problems of children with respect to differences in body size attitudes and cooperativeness However an all-encompassing presentation of the subject was never the author s intention His objective was to summarize the interesting results obtained by the group with which he collaborated Another objective was to present the noninvasive methods extensively employed by this group It is interesting and typical of the situation in Great Britain to note the attention devoted to ethical considerations Ethical problems were also the main reason why the group sought new and indirect procedures in this particular field

The book s different chapters deal with ways to measure the response to exercise and determine cardiac output (especially the indirect CO_2 rebreathing method) The response of normal children to exercise and the response of children with heart and lung disease to exercise are also considered A major part of the book is devoted to explaining and promoting the CO_2 rebreathing method (=indirect Fick Method) in the determination of cardiac output CO_2 production (V_{CO_2}) and the mixed venous–arterial CO_2 difference must be known in this method CO_2 is measured in the conventional manner by determining the CO_2 content of expired air The mixed venous CO_2 content is determined by means of CO_2 rebreathing and arterial CO_2 levels by sampling arterialized ear lobe blood

The method is based on the assumption that alveolar and arterial CO_2 pressures are equal This is usually the case However I do feel that this method should be used with caution until the assumption has been proved to be valid This is especially true in subjects with right to left shunts as in Fallot s tetralogy When arterialized capillary blood is used instead of arterial blood as in these studies there is a risk of introducing another source of error On the other hand the CO_2 rebreathing method displayed good reproducibility in the hands of Godfrey et al and there is also close agreement with more direct methods such as the direct Fick method and the dye dilution method in normal children

Perhaps the most interesting part of the books is devoted to exercise in the asthmatic child The emphasis here is on exercise induced asthma (EIA) The most important finding was that running provokes EIA more readily that cycling And the fact that swimming was even less provocative may have great practical significance The explanation for these differences could not be found However the impression was that this English team plans to have another go at the problem

This book provides considerable information and data for people interested in paediatric exercise physiology The book is also of value to those interested in learning about paediatric exercise physiology Limiting the presentation to the aspects of paediatric exercise physiology studied by the author s group is no serious shortcoming However a more comprehensive presentation is to be hoped for in future editions of the book

Bengt Eriksson

S Z Walsh W W Meyer & J Lind *The human fetal and neonatal circulation Function and structure* 351 pp illus Charles C Thomas Springfield Ill 1974 US $15 00

This book is a general review of the dramatic circulatory adaptation of the neonate The results of 20 years studies of the human fetal and neonatal period from the Wenner Gren Cardiovascular Research Laboratory in Stockholm from the basis for this extensively illustrated monograph It is the cooperation between physiology and morphology that has made this book unique The physiological aspects of the cardiovascular adaptation of the newborn are given in relation to the anatomical findings and histological structures are illustrated with excellent and beautiful anatomical and histological pictures

The interrelation between function and structure during the rapid growth and development of the circulatory system after birth is the main theme of the book The morphological studies of the vessels have helped the authors in the evaluation of some aspects of circulatory adaptation One can agree with the authors that much of the morbidity and mortality in the perinatal period is the result of failure by the newborn infant to achieve this adaptation

Not a single animal experiment but only studies on human beings foetuses as well as newborns of different gestational age constitute the basis for the authors own studies Many original data and figures are presented in the book

In the section dealing with the fetal circulation the placental morphology and vascular tree are thoroughly presented with microangiography and scanning electron

microscopy illustrations There is a brief review of the amniotic fluid which is of increasing clinical interest The umbilical cord is presented with very detailed original figures of anatomy and histology The pathogenesis of the leucocytic infiltration found in normal cases is discussed and the authors present a new theory related to the rapid slowing of the blood stream through the umbilical vein during delivery

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Georg Theander

ANNOUNCEMENTS

INTERNATIONAL CONGRESS OF PAEDIATRIC ENDOCRINOLOGY

An International Congress of Paediatric Endocrinology will be held in Milan (Italy) September 30th to October 1st 1976

Plenary sessions and round tables will be organized on the following topics

Hypoglycemia

Glucagon

Growth hormone

Congenital adrenal hyperplasia

Official language English

Further information may be obtained from G Chiumello Centro di Endocrinologia Infantile Clinica Pediatrica IV dell Università di Milano Ospedale L Sacco Via G B Grassi 74 20157 Milano (Italy)

INTERNATIONAL STUDY GROUP OF DIABETES IN CHILDREN AND ADOLESCENTS

Founded last September in Paris The International Study Group of Diabetes in Children and Adolescents held its first meeting at Beilinson Medical Center Petah Tiqwa Israel on April 27th 1975 in conjunction with the 3rd Beilinson Symposium on Juvenile Diabetes

The aim of this group of world famous specialists and pioneers in the field of juvenile diabetes representing twelve countries is to promote collaboration in research on diabetes in young patients Eligibility for membership is limited to pediatricians and paramedical personnel who treat children and adolescents exclusively

The Steering Committee is as follows President Dr Henri Lestradet France Secretary General General Dr Zvi Laron Israel Treasurer Dr Helmut Loeb Belgium

An Advisory Committee was elected and committees were formed to study problems in six major areas of juvenile diabetes reports to be submitted at the next meeting of the Study Group which will convene next fall in Belgium Council members are Dr Oystein Aagenaes Norway Dr Giuseppe Chiumello Italy Dr Alan Drash United States Dr Rene François France Dr Jorge Sires Argentina and Dr Bruno Weber Germany

International Study Group of Diabetes in Children and Adolescents

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Acta Pædiatr Scand 64 785–789 1975

CAUSES OF DEATH IN TRANSPOSITION OF THE GREAT ARTERIES

A Clinical and Autopsy Study of 140 Cases

B LANDTMAN I LOUHIMO J RAPOLA and LEENA TUUTERI

From the Children s Hospital University of Helsinki Helsinki Finland

ABSTRACT Landtman B Louhimo I Rapola J and Tuuteri Leena (The Children s Hospital University of Helsinki Helsinki Finland) Causes of death in transposition of the great arteries A clinical and autopsy study of 140 cases Acta Paediatr Scand 64 785 1975.—The causes of death were assessed in 140 infants and children with transposition of the great arteries studied clinically and post mortem Half of the children were under one month old Death occurred during the first year of life in 118 cases Balloon atrial septostomy and/or cardiac surgery were performed in 37 cases and 7 patients died following operations for extracardiac malformations Congestive heart failure was the most common single cause of death occurring in 109 cases All but 7 patients were cyanotic Extracardiac malformations were encountered in 39 patients and were considered a main cause of death in 22 of these Various infections, mostly pneumonia occurred in half of the cases Forty-one patients had vascular accidents in various organs These complications were more common in operated than in nonoperated cases Miscellaneous causes of death including hyaline membrane disease and/or pulmonary atelectases occurred in 30 patients The study illustrated the complex symptomatology and therapeutic problems presented by critically ill infants with transposition of the great arteries

KEY WORDS Transposition of the great arteries causes of death in congenital heart disease

The poor outlook of children with transposition of the great arteries is well documented In a review of 201 conservatively treated patients Sharer (19) stated that only 45 survived the first year of life and that most deaths occurred in the neonatal period and early infancy A mortality of 90% during the first year of life has been reported from some centers (6 9)

The introduction of balloon atrial septostomy (17) and subsequent hemodynamic correction (10) has dramatically changed the outlook for children with transposition These procedures are now being performed in most cardiac centers and as a result many patients are now leading a normal life

The natural history of transposition and the results of septostomy and surgical procedures have been reviewed by several authors In most of these studies however less attention has been paid to the assessment of the causes of death in conservatively and surgically treated cases The present study aims at shedding further light on this question by reviewing clinical and autopsy findings in 140 infants and children with transposition treated and lost at our hospital during a 28 year period

MATERIAL AND METHODS

Tran position of the great arteries occurred in 140 of 1 000 infants and children with congenital heart disease who came to autopsy at the Children s Hospital in Helsinki from 1947 to September 1974 One fourth of the patients with transposition were seen within the past 4 years Since

Table 1 *Clinical and autopsy data on 140 infants and children with transposition of the great arteries*

The figures indicate numbers of patients

Age	Total	Sex M	Sex F	Heart failure	Cyanosis	Non cardiac malformations	Infections	Vascular accidents	Various disorders	Septostomy and/or cardiac surgery	Other operations
Under 7 d	36	22	14	23	34	13	14	11	13	10	5
7 d–1 m	29	16	13	25	26	7	16	8	5	4	1
1–3 m	25	20	5	22	25	7	15	6	3	6	1
3–6 m	23	13	10	19	22	7	13	6	2	4	
6 m–1 yr	5	3	2	4	4	1	4			2	
1–3 yr	15	10	5	11	15	2	8	8	3	9	
3–5 yr	2	1	1	1	2	1	2				
5–12 yr	5	4	1	4	5	1	1	2	4	2	
Total	140	89	51	109	133	39	73	41	30	37	7

1967 balloon atrial septostomy has been performed in 61 cases A total of 121 infants and children with transposition have undergone this procedure and/or cardiac surgery at the hospital

The definition of transposition was based on the main criteria suggested by Paul & van Praagh (16) Transposition with the aorta arising from the right ventricle and the pulmonary artery from the left occurred in 122 of the cases Eleven patients had a double outlet right ventricle and 7 had transposition with a single ventricle Needless to say the anatomical differentiation of transposition from certain other complicated cardiac malformations was sometimes a question of interpretation

Information on the patients was collected from the autopsy reports and from the clinical records made on the children during treatment at the hospital The assessment of the immediate cause of death was not always easy Indeed the causes were frequently multiple particularly in the neonates and young infants In presenting the results reference will therefore be made only to significant clinical findings and major pathological changes without any attempt being made to separate primary from secondary causes of death in individual cases Thirty seven patients died following balloon atrial septostomy and/or cardiac surgery and 7 died after emergency operations for extracardiac malformations The causes of death did not differ significantly in the children with classical transposition a double outlet right ventricle and transposition and a single ventricle Hence the three groups will be discussed together

RESULTS

Some main clinical and autopsy data on the 140 patients are summarized in Table 1 There were 89 boys and 51 girls Moderate or severe asphyxia at birth occurred in 72 of the cases Thirteen patients had a birth weight of less than 2500 g The remaining children were comparatively heavy at birth (average 3390 g) but most of them showed marked retardation in growth, their weight at the time of death being below the 2 5th percentile in 44% of the cases Hence a poor general condition was a common contributory cause of death

Death occurred during the first year of life in 118 cases (84%) Almost half of the patients were under one month and one fourth less than 7 days old Five patients 2 of whom had a single ventricle lived for more than 5 years The oldest patient was 12 years old

Associated cardiac anomalies were common Ventricular and atrial septal defects occurred in 87 and 27 cases respectively A patent ductus arteriosus was present in 79 cases Nineteen patients had pulmonic stenosis Additional cardiovascular anomalies were encountered in 35 patients These included coarctation of the aorta (8 cases) anomalies of the venous return (8 cases) and solitary cases of aortic and mitral stenosis endocardial fibroelastosis dextrocardia common atrioventricular canal single coronary artery hypoplastic left heart syndrome tricuspid atresia etc Most of the patients with these complicated lesions died during the neonatal period

Congestive heart failure was a common clinical finding and a main cause of death in 109

cases (Table 1) All but 7 patients were cyanotic Pathological heart murmurs were often lacking in the neonates and occurred in 65% of all cases

Single or multiple extracardiac malformations were encountered in 39 patients (Table 1) These lesions were considered a main cause of death in 22 cases The anomalies were confined to the central nervous system in 7 and to the lungs in 3 cases Esophageal atresia (4 cases) and anal atresia (5 cases) were the most common malformations of the alimentary tract encountered in 16 patients Eleven patients had anomalies of the urinary system including hydronephrosis in 5 and agenesis of the kidney in 2 cases Cleft palate (4 cases) and anomalies of the spine (5 cases) were the most common of 16 malformations of the skeleton One patient had choanal atresia Miscellaneous anomalies (18 cases) included situs inversus in 5 and asplenia in 4 cases One patient had Down s syndrome Major extracardiac malformations were rare in the older children Thus of 22 patients over one year of age only 4 had minor anomalies of other organs

Various infections contributed to the fatal outcome in 73 of the cases (Table 1) Pneumonia occurred in 58 patients Other infections included gastroenteritis in 6 and septicemia in 5 cases One patient had a brain abscess and one had carditis

Vascular accidents (hemorrhages and thromboembolism) occurred in 41 patients (Table 1) *These lesions were encountered* in the brain in 20 in the lungs in 16 and in abdominal organs in 10 cases One patient had cardiac infarction

Various diseases were causes of death in 30 cases These included hyaline membrane disease and/or atelectases of the lungs (15 cases) and solitary cases of nephropathies hemolytic disease of the newborn cerebral lesions intussusception etc One child had a renal tumour Five patients died following perforation of the heart during cardiac catheterization

Balloon atrial septostomy and/or cardiac surgery were performed on 37 patients Thirteen patients had balloon septostomy only A Mustard operation was performed on 5 patients and 15 underwent shunt procedures or operative septostomy Death occurred within a week after septostomy or surgery in 31 cases Two of the patients had a double outlet right ventricle and 3 had transposition with a single ventricle Cardiac anomalies in addition to septal defects patent ductus arteriosus and pulmonic stenosis were encountered in 6 cases Table 1 shows the age of the patient at the time of death Twenty were under 3 months old Extracardiac malformations were present in 8 patients but in only one was the anomaly considered a main cause of death Vascular accidents were comparatively common occurring in 18 of the cases The incidence of heart failure and other main causes of death was by and large the same in surgically and conservatively treated cases An additional 7 patients died following operations for extracardiac malformations

DISCUSSION

Mortality statistics in transposition of the great arteries are not readily comparable because of differences in methods employed in published reports Although the present study comprised a selected group of patients it illustrates the complex symptomatology and therapeutic problems presented by critically ill infants with this cardiac malformation

Transposition occurred in 14% of 1000 cases of congenital heart disease in the present autopsy series The incidence of transposition in autopsy series of congenital heart disease varies in reports from some other pediatric centres between 6 7 and 20 8% (4 6 7 11 18) There is no evidence that the overall incidence of transposition in Finland has significantly changed during the time period covered by the study One fourth of the patients were seen during the past 4 years but since the introduction of balloon atrial septostomy in

Table 1 *Clinical and autopsy data on 140 infants and children with transposition of the great arteries*
The figures indicate numbers of patients

Age	Total	Sex M	Sex F	Heart failure	Cyanosis	Non cardiac malformations	Infections	Vascular accidents	Various disorders	Septostomy and/or cardiac surgery	Other operations
Under 7 d	36	22	14	23	34	13	14	11	13	10	5
7 d–1 m	29	16	13	25	26	7	16	8	5	4	1
1–3 m	25	20	5	22	25	7	15	6	3	6	1
3–6 m	23	13	10	19	22	7	13	6	2	4	
6 m–1 yr	5	3	2	4	4	1	4			2	
1–3 yr	15	10	5	11	15	2	8	8	3	9	
3–5 yr	2	1	1	1	2	1	2				
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1967, an increasing number of newborns suspected of having transposition are being referred to our hospital

The present study supports a common observation according to which transposition shows a male preponderance and the birth weight of the patients is comparatively high (19) The order of birth on the other hand did not significantly differ from that of Finnish children in general (8) Associated cardiovascular anomalies were somewhat more common in the present series than in autopsied cases of transposition reported from some other pediatric centres (6 9 12 16 19)

Most authors agree that congestive heart failure augmented by a varying degree of hypoxia is the most common single cause of death in transposition Heart failure occurred in 78% of the patients in the present series and cyanosis was an almost constant finding In a review of 308 fatal cases of transposition Sharer (19) considered heart failure the primary cause of death in 90 and Liebman et al (9) assessed that failure accounted for death in 71 of 282 autopsied cases Surgical deaths were the most common in both these studies

Extracardiac causes of death were more common in the present than in many previous studies Malformations of other vital organs occurred in 39 patients Sharer (19) considered noncardiac anomalies a primary cause of death in only 2 of 308 cases of transposition and Liebman et al (9) did not mention that such malformations had contributed to the death of any of 282 patients studied post mortem

Several autopsy studies have shown an incidence of non-cardiac malformations in transposition of less than 5% (3 9 18 22) but an incidence of 23% was reported by Okada et al (13) in an autopsy study of 196 cases It should be mentioned that anomalies of other organs were less common in transposition than in other cardiac malformations in the present autopsy series (8)

Various infections contributed to the fatal outcome in half of the cases In some of the aforementioned studies infections mostly pneumonia were considered a main cause [of] death in 10 to 25% of the cases (4 9 15 19) [A] brain abscess occurred in one surgical case [in] the present series Sharer (19) reported th[is] complication in 4 of 409 autopsied cases [of] transposition

The occurrence of vascular accidents i[n] transposition is well recognized Oppenheime[r] (14) found such lesions in 37 of 180 case[s] studied post mortem Of 43 infants with transposition palliated by balloon atrial septostomy 5 developed cerebrovascular accidents and 7 others showed seizure activity during the follow up (1) Parsons et al (15) reported that 9 of 32 deaths following this procedure resulted from thrombosis mainly of the cerebral vessels The present study supports an observation according to which vascular accidents are more common in surgically than in conservatively treated patients with congenital heart disease (2 20 21) Various other disorders such as hyaline membrane disease and nephropathies are to our knowledge not mentioned as causes of death in transposition in previous reports

The outlook of children with transposition of the great arteries depends on both the morphology of the heart malformation and the occurrence of primary extracardiac diseases and complications These factors also govern the ultimate benefit derived from balloon atrial septostomy and cardiac surgery Hoffman (5) has recently reviewed the difficulties involved in assessing the immediate cause of death in fatal cases of congenital heart disease In order to show that death was due to the cardiac malformation only there should be supportive clinical evidence which however is lacking in many autopsy reports The high incidence of extracardiac causes of death in the present series suggests that such disorders might explain differences in mortality statistics and operative results in transposition of the great arteries

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REFERENCES

1 Baker F Baker L Zoltun R & Zuberbuhler J R Effectiveness of the Rashkind procedure in transposition of the great arteries *Circulation 43* 1 1971
2 Bertrong M & Sabiston D C Jr Cerebral lesions in congenital heart disease A review of autopsies on one hundred and sixty two cases *Johns Hopkins Med J 89* 384 1951
3 Boesen J Melchior J C Tersley E & Vendel S *Extracardiac congenital malformations in children* with congenital heart disease *Acta Paediatr Scand* Suppl 146 78 1963
4 Fontane R S & Edwards J E *Congenital cardiac disease A review of 357 cases studied pathologically* W B Saunders Philadelphia and London 1962
5 Hoffman J I E Natural history of congenital heart disease Problems in its assessment with special reference to ventricular septal defects *Circulation 37* 97 1968
6 Keith J D Rowe R D & Vlad P *Heart disease in infancy and childhood* MacMillan New York 1967 2nd ed
7 Lambert E C Canent R V & Hohn A R Congenital cardiac anomalies in the newborn A review of conditions causing death or severe distress in the first month of life *Pediatrics 37* 343 1966
8 Landtman B Clinical and morphological studies in congenital heart disease A review of 777 cases *Acta Paediatr Scand* Suppl 213 1971
9 Liebman J Collum L & Belloc N D Natural history of transposition of the great arteries Anatomy and birth and death characteristics *Circulation 40* 237 1969
10 Mustard W T Successful two-stage correction of transposition of the great vessels *Surgery 55* 469 1964
11 Nadas A S & Fyler D C *Pediatric cardiology* W B Saunders Philadelphia–London–Toronto 1972 3rd ed
12 Noonan A Nadas A S Rudolph A M & Harris G B C Transposition of the great arteries A correlation of clinical physiologic and autopsy data. *N Engl J Med 263* 592 1960
13 Okada R Johnson D & Lev M Extracardiac malformations associated with congenital heart disease *Arch Pathol 85* 649 1968
14 Oppenheimer E H Arterial thrombosis (? paradoxial embolism) in association with transposition of the great vessels *Johns Hopkins Med J 124* 202 1969
15 Parsons C G Astley R Burrows F G O & Singh S P Transposition of the great arteries A study of 65 infants followed for 1 to 4 years after balloon septostomy *Brit Heart J 33* 725 1971
16 Paul M H van Praagh S & van Praagh R Transposition of the great arteries *In* H Watson (ed) *Paediatric cardiology* Lloyd Luke London 1968 p 576
17 Rashkind W J & Miller W W Creation of an atrial septal defect without thoracotomy *JAMA 196* 991 1966
18 Rowe R D & Mehrizi A *The neonate with congenital heart disease* W B Saunders Philadelphia–London–Toronto 1966
19 Sharer R M *Complete transposition of the great arteries* Academic Press New York and London 1973
20 Terplan K L Patterns of brain damage in infants and children with congenital heart disease Association with catherization and surgical procedures *Am J Dis Child 125* 175 1973
21 Wigger H J Bransilver B R & Blanc W A Thrombosis due to catherization in infants and children *J Pediatr 76* 1 1970
22 Wiland O K Extracardiac anomalies in association with congenital heart disease Analysis of 200 necropsy cases *Lab Invest 5* 380 1956

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FANCONI S ANEMIA

II *Are Multiple Endocrine Insufficiencies a Substantial Part of the Disease?*

P STUBBE and G PRINDULL

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ABSTRACT Stubbe P and Prindull G (Department of Paediatrics University of Goettingen, D 34 Gottingen Kinderklinik BRD) Fanconi s anemia II Are multiple endocrine insufficiencies a substantial part of the disease? Acta Paediatr Scand, 64 790 1975 —Three children with Fanconi's anemia belonging to a family where 6 children had the disease were investigated One child had growth hormone deficiency a second child showed subnormal response of testosterone to gonadotropin stimulation and the third child had a missing insulin release following arginine This report shows that growth hormone deficiency is not necessarily linked with Fanconi s anemia when it occurs in a family Multiple endocrine insufficiencies do not appear to be part of the disease

KEY WORDS Fanconi s anemia hormones growth hormone

The typical features of Fanconi s anemia (FA) consists of small stature pancytopenia skeletal and other organ deformities skin pigmentation cryptorchidism and chromosomal breakage (3 10) Endocrine disturbances have long been thought to be linked with stunted growth and other symptoms of the disease Only two reports have been published in which evidence of isolated growth hormone deficiency was well documented (11 25) The present paper describes a family with FA where growth hormone assay and determinations of other hormones were performed in three children The diagnosis of FA in these children was established on the basis of hematological clinical and chromosomal findings and will be described in detail elsewhere in connection with various other laboratory studies (13)

METHODS AND PATIENTS

Radioimmunoassay of growth hormone (GH) and insulin (7 24) in the serum was performed after stimulation with i v arginine infusion (0 5 g/kg/30 min) and insulin induced hypoglycemia (0 1 U of regular insulin i v) on two successive days A NIH preparation was used as GH standard (NIH GH HS 1394) and the GH antiserum was a gift of Dr S A Berson No 2 5 19 New Yord Human insulin was used as standard The assays exhibit variations of 10% and have a lower limit of detection of 0 5 ng/ml and 1 μU/ml for GH and insulin respectively Plasma cortisol levels were measured at 8 a m and 7 p m and after ACTH application (9) Thyroxine and thyrobinding index were determined by using commercially available test kits (Abotts Tetrasorb 125 Amersham thyopac 3) 17 ketosteroids and total corticoid excretion were performed in 24 hour urines collected before and after metapirone administration (0 25 g every 4 h for 6 doses) (1 8) The luteinizing hormone releasing hormone test (LH RH test) was performed by injecting 25 micrograms LH RH (Hoechst) per 20 kg bodyweight and measuring LH before and 30 minutes after the injection (16) Testosterone (22) was determined before and after injection of human chorionic gonadotropin in a dose of 5000 I U /day on 5 consecutive days Blood glucose (hexokinase method) and urea were determined by using commercially available test kits (Boehringer) The bone age was determined by using Greulich Pyle s method (6)

Patients were identified in the same manner as will be described elsewhere (13) and were without medication Patients IV/6 IV/7 and IV/8 were 8 4/12 5 1/12 and 1 5/12 years of age respectively at the time the investigations were performed Except for anemia and moderate renal insufficiency in patient IV/8 the children were in good health at the time of study The procedures were carefully explained to the parents and their informed consent was obtained

Table 1 *Growth hormone (GH) insulin and blood glucose after arginine infusion and insulin induced hypoglycemia in 3 children with Fanconi s anemia*

Patient IV/6							
Time (min)							
Arginine	0	15	30	45	60	90	1 0
Insulin	0	10	0	30	40	60	1 0
Arginine							
GH (ng/ml)	14	1 5	3 3	8 2	21 8	9 7	3 6
Insulin (μU/ml)	4	33 5	43 2	13 2	4	<1	8
Glucose (mg/100 ml)	68	101	98	85	75	77	7[illegible]
Insulin							
GH (ng/ml)	[illegible] 6	2 9	3 4	4 7	9 2	7 1	4
Glucose (mg/100 ml)	68	46	[illegible]6	45	60	74	72
Patient IV/7							
Arginine							
GH (ng/ml)	11 3	15 7	12 [illegible]	17 7	18 2	7 5	4 8
Insulin (μU/ml)	<1	3	4	<1	<1	<1	<1
Glucose *(mg/100 ml)*	40	45	61	64	62	53	49
Insulin							
GH (ng/ml)	3 3	12 5	13 3	15 2	14 6	4 6	0 9
Glucose (mg/100 ml)	88	89	60	52	48	6[illegible]	7[illegible]
Patient IV/8							
Arginine							
GH (ng/ml)	<0 5	<0 5	0 7	<0 5	1 [illegible]	[illegible]	<0 5
Insulin (μU/ml)	<1	4 3	16	7 1	7	2 7	[illegible]
Glucose (mg/100 ml)	60	75	89	81	6[illegible]	46	49
Insulin							
GH (ng/ml)	1	<0 5	<0 5	<0 5	<0 5	<0 5	1 1
Glucose *(mg/100 ml)*	61	4[illegible]	30	16	27	9	46

RESULTS

Growth hormone

Insulin induced hypoglycemia was followed by a normal increase of GH in 2 patients reaching 9 2 ng/ml in patient IV/6 and 15 2 ng/ml in patient IV/7 (Table 1) The arginine infusion caused an increase of GH in patient IV/6 from a normal baseline level to 21 8 ng/ml whereas the probably stress induced and elevated GH concentration in patient IV/7 varied between 11 3 ng/ml initially and 18 2 ng/ml after 60 minutes (Table 1) In the third patient studied (IV/8) GH levels remained less than 2 ng/ml GH therapy was later instituted in this child (2×2 ml/week) but within 2 ½ months of therapy no improvement in growth or anemia could be achieved before the patient died

Insulin

Patients IV/6 and IV/8 showed a normal and low normal insulin secretion following arginine reaching peaks of 43 2 and 16 μU/ml respectively whereas patient IV/7 had no significant change of insulin concentration (Table 1)

Glucose

Following hypoglycemia patient IV/8 showed a delayed return of blood glucose to normal which still did not reach fasting levels after 120 minutes (Table 1)

Corticoids

Determinations of morning levels of plasma cortisol in three children and the diurnal variation in one showed normal concentrations between 11 1 and 24 6 μg/100 ml (Table 2) The response to i v administration of 0 25 mg synthetic beta 1–24 ACTH (Synacthen Ciba) was also normal (Table 2) The 17 ketosteroid excretion was low in two children with 0 094 and 0 005 mg/24 hours (Table 2) After metapirone administration there was no increase of corticosteroids in the urine

Table 2 *Thyroxine thyro binding index cortisol 17 ketosteroids 17 hydroxycorticosteroids testosterone luteinizing hormone urea retention test and urea in 3 children with Fanconi s anemia*

Patient	VI/6	VI/7	VI/8
Total serum thyroxine (μg/100 ml)	6 6	9 7	5 3
Thyro-binding index	0 97	1 2	1 18
Plasma cortisol (μg/100 ml)			
8 a m	24 6	11 1	16 8
7 p m	–	4 4	–
Plasma cortisol during ACTH test (μg/100 ml)			
Before	24 6	11 1	16 8
2 hrs after	43 2	42 8	30 2
Urinary 17 ketosteroid excretion (mg/24 hrs)	0 094	–	0 005
Metyrapone test 17 hydroxy corticosteroids (mg/24 hrs)			
Before	1 0	–	0 82
During	0 71	–	0 5
1 day after	0 4	–	0 71
Plasma testosterone before and after 5 day course of 5 000 IU gonadotropin daily (ng/100 ml)			
Before	42	–	–
After	98	–	–
LH during LHRH test (25 μg i v) (mIU/ml)			
Before	<1 25	–	<1 25
30 min after	4 29	–	<1 25
Urea retention during 5 day treatment with growth hormone (%)	16 5	5 5	21 5
Serum urea before and after 5 day treatment with 1 mg growth hormone (mg/100 ml)			
Before	27 5	41	99
After	27 9	26 6	50 1

– not done

Thyroid hormones

Thyroxine concentrations were between 5 3 and 9 7 μg/100 ml and normal in all 3 children The thyrobinding index was also normal (Table 2)

Luteinizing hormone

The LH RH test did not cause an increase in LH levels and remained lower than 1 65 m I U /ml in patient IV/8 The increase of LH in patient IV/6 was normal for this age group in reaching 4 29 m I U /ml after 30 min (Table 2)

Testosterone

The testicular function was investigated only in patient IV/6 who had no palpable testicles The increase from 42 to 98 ng/100 ml was less than the normal mean (329±172 ng/100 ml mean±S D) (14)

Urea retention

The urea retention test is simpler to perform than nitrogen retention and gives comparable results (12) All children were put on a constant diet for a 10-day period and received 1 mg of GH daily from the 6th to 10th day Patient IV/8 who had moderate renal insufficiency and a defective GH secretion showed a 21 5 per cent retention of urea during GH treatment whereas the other 2 patients had lower retention values with 16 5% and 5 5% respectively (Table 2) Serum urea concentrations decreased during GH treatment in patients IV/7 and IV/8 and remained unchanged in patient IV/6 (Table 2)

Somatic growth

Spontaneous growth and weight gains as well as bone age determinations of the children can be seen in Table 3 and Fig 1 Patient IV/8 who had GH deficiency had no increase in height

Table 3 *Spontaneous height and weight gain and bone age development in 3 children with Fanconi s anemia*

Patient IV/8 was treated with human growth hormone for 2½ months starting at the age of 2 4/12 years

Age (years/months)	Bone age (years/months)	Height (cm)	Weight (kg)
Patient IV/6			
5/7	2/0	90	8 8
7/1	–	98	11 8
8	–	102	13
9/2	5/0	106	15 5
Patient IV/7			
7/3	1/0	72	7 3
4	–	84	9
5/1	2/6	91	10 7
5/6	–	93 8	11
6/1	3/0	99	13 5
Patient IV/8			
0/2		47	2 6
1/2		53	4
1/5	<0/3	58	4 1
2/3	<0/3	61	4
2/7		61	3 8

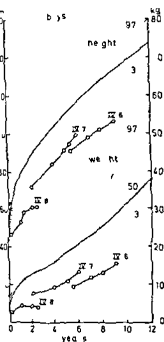


Fig 1 Spontaneous height and weight gain in 3 children with Fanconi s anemia The 3rd 50th and 97th percentile for healthy german boys is given for comparison Patient IV/8 was treated with human growth hormone for 2½ months starting at the age of 2 4/12 year

for at least 6 months whereas patients IV/6 and IV/7 grew 3 4 cm/year and 8 cm/year respectively without receiving GH treatment Bone age was grossly retarded to about half the chronological age (Table 3)

DISCUSSION

This report describes endocrine investigations in the largest family with FA that we know of We found GH deficiency and normal GH secretion in different members of the same family Only two other reports describing three patients with FA have been published where defective GH secretion was well established (11 25) Another abstract supplied limited information only (18) From the two reports it remained unclear whether FA is always associated with GH deficiency when it occurred in the same family One out of 3 children in the family under discussion was GH deficient when the secretion was investigated with arginine or insulin induced hypoglycemia

This family documented that FA is not necessarily linked with GH deficiency Azotemia and renal acidosis were permanently present in the GH deficient child and were probably responsible for the lack of success of GH therapy in this child Uremia is known to be associated with increased basic GH levels and consequently cannot be made responsible for the very low GH secretion (15 23) Haemosiderosis as an explanation of endocrine dysfunction cannot be applied to the GH deficient child since the boy had received only a total of 50 ml red cell concentrate (20) Except for the deficient secretion of GH in one child no complete failures of other endocrine functions were observed in this family Incomplete failures on the other hand were common as have been shown by others Determinations of 17 ketosteroids in patients with FA have been found to be decreased or normal (2 4 5 19) The missing response to metapirone has to be interpreted as a decreased ACTH reserve provided the substance was absorbed readily The insulin levels were subnormal in one child We are not aware of insulin determinations in patients with FA and have not investigated insulin secretion with other procedures Cryptorchidism has frequently been noticed in patients with FA but investigations of endocrine testicular functions have not been reported The subnormal increase of testosterone after stimulation with human chorionic gonadotropin pointed toward Leydig cell insufficiency (14) The LH response to synthetic LH RH was within normal limits according to our own data (21) Summarizing the findings of endocrinological investigations we do not think that multiple endocrine insufficiencies are necessarily associated with Fanconi s anemia Treatment of FA patients with GH even without GH deficiency was unsuccessful (17) It thus appears that the therapeutic use of GH in patients with FA is to be limited to those where proven GH deficiency exists

Table 2 *Thyroxine thyro binding index cortisol 17 ketosteroids 17 hydroxycorti costeroids testosterone luteinizing hormone urea retention test and urea in 3 children with Fanconi s anemia*

Patient	IV/6	IV/7	IV/8
Total serum thyroxine (μg/100 ml)	6 6	9 7	5 3
Thyro binding index	0 97	1 2	1 18
Plasma cortisol (μg/100 ml)			
8 a m	24 6	11 1	16 8
7 p m	–	4 4	–
Plasma cortisol during ACTH test (μg/100 ml)			
Before	24 6	11 1	16 8
2 hrs after	43 2	42 8	30 2
Urinary 17 ketosteroid excretion (mg/24 hrs)	0 094	–	0 005
Metyrapone test 17 hydroxy corticosteroids (mg/24 hrs)			
Before	1 0	–	0 82
During	0 71	–	0 5
1 day after	0 4	–	0 71
Plasma testosterone before and after 5 day course of 5 000 IU gonado tropin daily (ng/100 ml)			
Before	42	–	–
After	98	–	–
LH during LHRH test (25 μg i v) (mIU/ml)			
Before	<1 25	–	<1 25
30 min after	4 29	–	<1 25
Urea retention during 5 day treat ment with growth hormone (%)	16 5	5 5	21 5
Serum urea before and after 5 day treatment with 1 mg growth hormone (mg/100 ml)			
Before	27 5	41	99
After	27 9	26 6	50 1

– not done

Thyroid hormones

Thyroxine concentrations were between 5 3 and 9 7 μg/100 ml and normal in all 3 children The thyrobinding index was also normal (Table 2)

Luteinizing hormone

The LH RH test did not cause an increase in LH levels and remained lower than 1 65 m I U /ml in patient IV/8 The increase of LH in patient IV/6 was normal for this age group in reaching 4 29 m I U /ml after 30 min (Table 2)

Testosterone

The testicular function was investigated only in patient IV/6 who had no palpable testicles The increase from 42 to 98 ng/100 ml was less than the normal mean (329±172 ng/100 ml mean±S D) (14)

Urea retention

The urea retention test is simpler to perform than nitrogen retention and gives comparable results (12) All children were put on a con stant diet for a 10-day period and received 1 mg of GH daily from the 6th to 10th day Patient IV/8 who had moderate renal insuffi ciency and a defective GH secretion showed a 21 5 per cent retention of urea during GH treatment whereas the other 2 patients had lower retention values with 16 5% and 5 5% respectively (Table 2) Serum urea concentra tions decreased during GH treatment in pa tients IV/7 and IV/8 and remained unchanged in patient IV/6 (Table 2)

Somatic growth

Spontaneous growth and weight gains as well as bone age determinations of the children can be seen in Table 3 and Fig 1 Patient IV/8 who had GH deficiency had no increase in height

Table 3 *Spontaneous height and weight gain and bone age development in 3 children with Fanconi s anemia*

Patient IV/8 was treated with human growth hormone for 2½ months starting at the age of 2 4/12 years

Age (years/months)	Bone age (years/months)	Height (cm)	Weight (kg)
Patient IV/6			
5/7	2/0	90	8 8
7/1	–	98	11 8
8	–	102	13
9/2	5/0	106	15 5
Patient IV/7			
2/3	1/0	72	7 3
4	–	84	9
5/1	2/6	91	10 7
5/6	–	93 8	11
6/1	3/0	99	13 5
Patient IV/8			
0/2		47	2 6
1/2		53	4
1/5	<0/3	58	4 1
2/3	<0/3	61	4
2/7		61	3 8

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HEALTH SCREENING OF FOUR YEAR OLDS IN A SWEDISH COUNTY

I *Organization Methods and Participation*

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ABSTRACT Sundelin C and Vuille J C (Department of Paediatrics University Hospital Uppsala Sweden and the Institute of Social and Preventive Medicine University of Berne Berne Switzerland) Health screening of four year olds in a Swedish county I Organization methods and participation Acta Paediatr Scand 64 795 1975.—Since January 1969 a systematic health screening has been offered to 4 year-olds resident in the county of Uppsala Sweden at the ordinary Child Health Centres by the regular staff This report describes some organizational and methodological aspects and presents participation rates and reasons for non participation From the overall experience it is concluded that the program is feasible with respect to systematic examinations and data collection acceptance by the public and the staff and with respect to the strain on existing resources The reasons for and the importance of program changes are discussed and the main advantages and drawbacks of the decentralized approach are mentioned Finally a theoretical framework for a quantitative evaluation of effectiveness is presented

KEY WORDS Pre school children health screening organization methods evaluation

In 1968 the Swedish National Board of Health and Welfare published a recommendation to the local authorities to start and maintain a general health survey of all 4 year olds according to a program which the Board elaborated in detail (3)

Health screening may either be performed by specialized screening centres (1) or incorporated into a traditional system of health care provision In the county of Uppsala (as in almost every county) the program was intended to be incorporated into the existing system of Child Health Care and to be carried out by the ordinary staff of the Child Health Centres (CHC) The program was initiated in January 1969 and since January 1971 all 4 year olds resident in the county have had the possibility of undergoing the check up

The present article deals with the organization methods and participation rates as well as reasons for non participation The purpose is to present necessary background information for subsequent papers concerned with the effectiveness of the health screening program in the hands of the ordinary CHC staff

ORGANIZATION

The primary examinations were performed by a number of different teams consisting of a nurse a physician and auxiliary staff The nurses were either specialized in child health care and working full time in CHC s of type I (urban areas) or public health nurses with many other duties in the community (rural areas CHC type II) The 4? participating physicians were either trained pediatricians young physicians in the course of pediatric training or district doctors (general practitioners) most of them devoting only a small part of their time to preventive pediatrics Because of considerable mobility among the

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This study was supported by the Deutsche Forschungs gemeinschaft Bonn-Bad Godesberg W Germany Cortisol and LH were determined by Dr Koebberling and Dr v z Muehlen Department of Internal Medicine Urinary 17 ketosteroids corticoids and testosterone were done in the Department of Experimental Endocrinology by Dr A Koenig and Dr F Elsaesser The human insulin used as standard was a gift of Dr O Jacobi Novo Industrie GmbH Mainz Arginine was kindly supplied by B Braun Melsungen This study was presented in part at the 70th Meeting of the German Paediatric Society Nurnberg 1973

REFERENCES

1 Appleby J I Gibson G Norymberski J K & Stubbs R D Indirect analysis of corticosteroids I The determination of 17 hydroxycorticosteroids *Biochem J 60* 453 1955
2 Cowdell R H Phizacherley P J R & Pyke D A Constitutional anemia (Fanconi s syndrome) and leukemia in two brothers *Blood 10* 788 1955
3 Fanconi G Familial constitutional panmyelopathy Fanconi s anemia (F A) I Clinical aspects *Semin Hematol 4* 233 1967
4 Francis R C Moir R A & Swift P N The value of splenectomy in Fanconi s anemia *Arch Dis Child 30* 439 1955
5 Gelli G Pancitopenia ipoplastica familiare tipo Fanconi (Anemia di Fanconi) *Minerva Pediatr 11* 1225 1957
6 Greulich W W & Pyle S I *Radiographic atlas of skeletal development of the hand and wrist* Stanford University Press Stanford Calif 1959 2nd edition
7 Herbert V Lau K L Gottlieb C W & Bleicher S J Coated charcoal immunoassay of insulin *J Clin Endocrinol Metab 25* 1375 1965
8 Jørgensen M On the determination of 17 ketogenic steroids in urine *Acta Endocrinol (Kbh) 26* 424 1957
9 Kobberling J & Muhlen A v z Methodische Untersuchungen zur Bestimmung der Plasmacorticoide mit der Proteinbindungsmethode *Z Klin Chem Klin Biochem 10* 67 1972
10 Nilsson L R Chronic pancytopenia with multiple congenital abnormalities (Fanconi s anemia) *Acta Paediatr Scand 49* 518 1960
11 Pochedly C Collipp P J Wolman S R Suzansinkul S & Rezvani I Fanconi s anemia with growth hormone deficiency *J Pediatr 79* 93 1971
12 Prader A Zachmann M Poley J R & Illig R The metabolic effect of a small uniform dose of human growth hormone in hypopituitary dwarfs and in control children *Acta Endocrinol (Kbh) 57* 115 1968
13 Prindull G Stubbe P & Kratzer Z Fanconi s anemia I Case histories clinical and laboratory findings in 6 siblings *Z Kinderheilkd* in press
14 Rivarola M A Bergodá C Cullen M & Calettí E HCG stimulation test in prepubertal boys with cryptorchidism in bilateral anorchia and in male pseudohermaphroditism *J Clin Endocrinol Metab 31* 526 1970
15 Saenger P Wiedemann E Korth Schutz S Lewy J E Riggio R R Rubin A L Stenzel K H Schwarts E & New M I Serum somatomedin (SM) and growth in chronic renal failure and after renal transplantation *Acta Paediatr Scand 63* 329 1974
16 Saxena B B Leyendecker G Chen W Gandy H M & Person R E Radioimmunoassay of folliclestimulating (FSH) and luteinizing (LH) hormones by chromatoelectrophoresis *Acta Endocrinol (Suppl) (Kbh) 14?* 185 1969
17 Scharer K & Baumann T Die Behandlung der idiopathischen Panmyelopathien mit Steroiden und hypophysarem Wachstumshormon *Schweiz Med Wochenschr 94* 1322 1964
18 Schonberg D Plasma growth hormone in cerebral gigantism Laurence-Moon-Bardet-Biedl syndrome Bloom syndrome and Fanconi anemia *Acta Paediatr Scand 62* 111 1973
19 Shahidi N T & Crigler J F Evaluation of growth and of endocrine systems in testosterone-corticosteroid treated patients with aplastic anemia *J Pediatr 70* 233 1967
20 Steel C M Butterworth C T G & Keay A J Endocrine studies in a case of congenital (erythroid) hypoplastic anemia *Acta Haematol (Basel) 47* 109 1972
21 Stubbe P Muhlen A v z & Heidemann P Die diagnostische Anwendung von LH RH im Pra pubertatsalter *Monatsschr Kinderheilkd* in press
22 Winter J S D & Grant D R A rapid and sensitive assay for plasma testosterone in adults and children *Anal Biochem 40* 440 1971
23 Wright A D Lowy C Russel Fraser T Spitz I M Rubenstein A H & Versohn I Serum growth hormone and glucose intolerance in renal failure *Lancet II* 798 1968
24 Wool M S & Selenkow H A Charcoal Dextran radioimmunoassay of human growth hormone *Acta Endocrinol (Kbh) 57* 109 1968
25 Zachmann M Illig R & Prader A Fanconi s anemia with isolated growth hormone deficiency *J Pediatr 80* 159 1972

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Table 2 *Participation at check up*[a]

Figures in parentheses are percentages of those who were resident in the respective area at the time of invitation

	1969	1970	1971	Whole period
Number of invitations mailed	1 051	2 951	3 540	7 542
Number of children still resident in respective area	1 0 0 (100 0)	2 877 (100 0)	3 444 (100 0)	7 341 (100 0)
Number of participants	992 (97 3)	2 818 (97 9)	3 363 (97 6)	7 173 (97 7)
Complete examination	917 (89 9)	2 699 (93 8)	3 077[b] (89 3)	6 693 (91 ?)
Partial examination	75 (7 4)	119 (4 1)	86 (8 3)	480 (6 5)
Number of non participants	?8 (? 7)	59 (2 1)	81 (? 4)	168 (2 3)
Main reason other health control	6 (0 6)	15 (0 5)	15 (0 4)	36 (0 5)
no interest	?? (? ?)	37 (1 3)	53 (1 5)	112 (1 5)
no possibility	0 (0 0)	7 (0)	13 (0 4)	0 (0 3)

[a] The median age at examination was 4 0 years in all three study years The 10th and 90th percentiles were 3 91 and 4 23 respectively a range which appears sufficiently narrow to allow the group to be considered as homogeneous with respect to age

[b] If an interview was not performed because it was considered unnecessary for the reasons given in the text the examination was classified as complete if no other items were missing

The category other health control applies in those cases where no other explanation is obvious

The nurses interview was by far the most time consuming item and in view of the serious shortage of staff we were forced to find a way to reduce the workload An analysis based on the data gathered in 1969 and 1970 showed that 93^(?) (?59 out of 278) of the children who were considered to need a further con ultation because of psychological problems could have been identified on the basis of the questionnaire and the result of the direct examination of the child The value of the interview as a screening instrument was found to be related primarily to its capacity to add more specific and precise information in those cases where other methods had revealed the existence of some problems (8) We therefore decided that from 1971 onwards the interview should be performed only for those cases where the other sources of information gave rise to suspicion of a significant problem as well as for all those children who were not previously known to the CHC or who were known to have presented behavioral problems before Concerning the rest of the children the nurses were free to decide whether they needed to carry out an interview or not

(b) Only three items in the motor test instead of eleven Concerning *motor skills* an analysis of the first 1000 cases showed that a combination of examination of gait and balance (walking on a plank 7 cm wide and ? m long) and of the use of scissors in cutting a piece of paper had a high probability of detecting those cases with motor dysfunction who were identified by the complete test of 11 items (8)

(c) Procedure for the detection of orthopedic anomalies simplified According to the original proposal the skeleton of every child had to be examined closely with—among other things—a systematic assessment of the functional range of the major joints The yield of the rather time consuming procedure was low and from 1971 the instruction was therefore given to look at the naked child from the back and laterally when standing and to observe possible changes in configuration ... when the child was walking

(d) Evaluation of speech development now performed by auxiliary staff with a ten word test The referral rates for speech anomalies during the first 2 years were much lower than expected on the basis of experience at other centres (Lindholm personal communication) Therefore a simple ten word test was elaborated and auxiliary personnel trained in the application of this test

(e) Ability of the child to understand and participate in the vision screening test included in the assessment of mental development North (7) in his review on research issues in Head Start put forward the hypothesis that a vision test in a preschool child might be more important as a means for detecting defects in mental capacity than as a method for discovering true health problems caused by disturbed visual functions The change in our program was made with the main purpose to test this hypothesis

(f) Substantial reduction of the number of forms to be completed by the staff

(g) A number of criteria concerning which children should be referred to a specialist were added to the data processing program The data of every child who satisfied one of these criteria but was not referred were re evaluated by the Chief Medical Officer often in collaboration with the examining team and as a rule either re examination or referral was recommended

(h) A comprehensive manual with detailed instructions was distributed

PARTICIPATION

It should be noted that there is no formal obligation for participation The participation rates as well as the main reasons for non participation as stated by the responsible nurses are shown in T ble ? The rates were remarkably stable throughout the 3 year period in pite of the fact that new regions of the county and new staff were gradually introduced and in spite of changes in details of the pro-

Table 1 *The screening program*

Source of information/ Examination	Performed by	Comment
Questionnaire	Parents	Replacing conventional pediatric history Contains a few very simple questions about the child s mental and emotional development
Interview concerning child s behaviour and development	Nurse	See text
Assessment of mental development	Nurse/Physician	Draw a man count 3 objects participation at examination observed behaviour
Test of speech development	Nurse/Physician Auxiliary	1969–1970 Simple observation (conversation) Since 1971 specific test Pronunciation of 10 key words (pictures)
Hearing test	Auxiliary	Audiogram at 250 500 1 000 2 000 4 000 and 8 000 c/s Pass level =25 dB
Vision test	Nurse/Auxiliary	Single symbol Snellen s E or Bostrom s hook Each eye tested separately
Test for bacteriuria	Auxiliary	Uriglox*
Height and weight	Auxiliary	
Physical examination	Physician	Special emphasis on motor coordination 11 items in 1969–70 3 items since 1971
Dental examination	Dentist	Orthodontic abnormalities Caries Oral hygiene and gingival disease

* Will not be discussed in the present context

physicians many teams were constituted *ad hoc* for only a limited period of time The calling routine using the computerized population register as a basis as well as the surveillance and data processing systems have been described elsewhere (7)

METHODS

In accordance with the proposal by the National Board of Health and Welfare the program is composed of the items shown in Table 1 [1]

Training of personnel for the special purpose

When the screening program was started many nurses and doctors were rather unfamiliar with some of the procedures especially the assessment of the developmental level and the systematic investigation of the child s emotional adjustment Since it was not practical to let all staff participate in full time courses as would have been desirable we chose the model of continuous education in small groups and repeated short sessions Unfortunately it has only been possible to arrange this training program for nurses and auxiliary staff whereas doctors were given only a short introductory lecture together with detailed printed instructions Whenever necessary this information has been completed by personal discussions concerning individual cases

Special examinations

If at the primary examination assessment and/or treatment by a specialist was deemed necessary the child was referred to the appropriate outpatient department at Uppsala University Hospital For general pediatric and psychological problems a special unit staffed by a pediatrician a child psychiatrist and a psychologist has been created Children with eye problems were treated by two ophthalmologists in the city of Uppsala one of them having acquired special training in pediatric ophthalmology All specialists have been asked to report the diagnosis and to give a semiquantitative estimation of the functional importance of the detected anomaly as follows

0 No anomaly or no need for treatment
1 Treatment recommendable but not absolutely necessary
2 Treatment necessary in order to relieve present suffering or to prevent probable significant impairment in the future
3 Definite handicap the consequences of which may be relieved by active intervention
C Further observation necessary Definite conclusion not yet possible

Children who were referred for problems already known about and adequately treated before were classified as 0 since no new action had resulted from the check up This information submitted by the specialists was added to the data file used for the present analysis

Changes in the screening program

The experience gained during 1969 and part of 1970 (8) was utilized to develop more efficient simpler routines which were introduced from the beginning of 1971 These changes included the following

(*a*) Interview to be performed in selected cases only

[1] An English version of the manual is available from the authors on request

neglected in the sense of inadequacy of health care provided for them but they seem to represent a small minority among the already small group of non participants It also appeared somewhat reassuring that more than half of the children had some other form of health supervision at 4 years of age There was documented evidence that not more than 4 children out of a total of 2 877 had never been seen at a CHC nor had had health supervision at the age of 4 years (Table 5)

COMMENTS

Quantitative data concerning the results obtained with this screening program will be presented in subsequent papers However a few issues must be discussed in the present context

1 *Feasibility*

The experience gained during the first 3 years has shown that the implementation of a systematic screening program into a pre existing health service is possible Data could be collected in a systematic way for every child concerned the procedure was accepted by the population (high participation rate) and the staff and the increase in the number of permanent staff necessary to compensate for the extra work load was acceptable (not more than 10%) Special examinations and treatment could be offered to all children needing them

2 *Significance of program changes*

Since routine health care rarely offers the opportunity for an experimental design the decision for changes in the program may not only be prompted by quantitative evidence—as in the case of the interview and the test of motor performance (see page 797)—but also by definite opinion of the primary staff (e g simplification of data collection procedure) or of directly involved specialists (new test for speech development) and by other sources as well (literature etc) Changes likely to bring about an improvement cannot be postponed until all the planned quantitative evaluations have been performed Such intervening changes may therefore represent a source of variation in any analysis of the total material In the ensuing publications attention will be paid to the possible effects of these changes

3 *The decentralized approach*

The main advantages of the approach advocated by the Swedish National Board of Health and Welfare are the preservation of the continuity of care the high degree of accessibility and the improvement in the professional competence of the staff—especially the nurses—performing the examinations On the other hand the large number of persons involved implies a substantial risk of suboptimal or even definitely poor performance This aspect of inter observer variability will be dealt with in paper III (6)

4 *Theoretical framework for the subsequent analysis*

In order to decide whether the advantages of the decentralized approach really outweigh the drawbacks it remains to be demonstrated that the overall performance of the program is acceptable The ultimate goal of the program obviously is to bring about an improvement of the health status of children through early detection and treatment of those pathologic conditions and developmental anomalies which are amenable to treatment (9) An attempt to demonstrate the program s effectiveness in achieving this goal would only be successful if a follow up study could show that the state of health of a cohort of schoolchildren who at the age of 4 years were given a chance to participate in the screening and to undergo the necessary treatments is better than the state of health of a group of control children Such a study is in progress in Uppsala but conclusive results will first be available in a couple of years In the meantime however some partial aspects of effectiveness should be analysed because such preliminary analyses may already point to areas which certainly need to be improved now Two important parameters of effectiveness are the sensitivity and the specificity of the screening process In order to compute quantitative estimates of these

Table 3 *Reasons for non participation in 54 children born in 1966*

Reasons for non participation	Total number	Other health supervision provided		No health supervision
		Total	Including vision screening	
Severely handicapped	7	7		0
Hospitalized	1	1		0
Check up considered unnecessary	17	15	7	2
Child afraid of doctors	7	1	1	6
No time available	10	4	1	6
Language difficulties	3	0	0	3
Overtly negative attitude	5	2	0	3
Abroad at time of check up	1	?		
No information	3	?		
Total	54	30	9	20

gram The very high rate of participation was achieved primarily thanks to the basically positive attitude of the general population towards the CHC as such (4) and the strong support given by all mass media and relevant au thorities Furthermore the nurses did their best to persuade hesitant parents by sending out a new invitation and by personal contact on the telephone and by home visits

201 children (2 7%) were still registered in the official population register though they had in fact already moved when the invitation was sent out It is not known whether these children were subsequently taken care of at their new place of residence nor whether this migrating popula tion represents a special risk group

For another 168 (2 3%) participation was refused by the parents for various reasons The hypothesis that these children constitute a group with special need of health supervision cannot be ruled out *a priori* (1) We therefore conducted a special study of non participants born in 1966 by collecting additional information from existing health records and by scrutinising the lists of patients currently treated at the Departments of Pediatrics Child Psychiatry Pediatric Surgery Orthopedics and Audiolo- gy and at the rehabilitation centres for motor and men tally handicapped children The parents of all those children whose records did not contain a satisfactory ex planation for their non participation were interviewed by a trained pediatric nurse not involved in the activities of the Child Health Centres Of the 59 non participants 5 were found to have had the check-up at a later date

Table 3 summarizes the main reasons for non participation in the remaining 54 cases Lack of interest or time were noted most often and for the majority of these children some other health supervision was provided The differences in background data (social class age marital status and gainful employment of mother arrangements for day-care) between non participants and the total ma terial were small and there was no evidence in favour of the hypothesis that non participants form a socially de prived risk group (Table 4) A few individual cases such as immigrants whose parents did not speak Swedish and those for whom no information could be obtained are probably to be looked upon as neglected children

Table 4 *Background data of 54 non participants*

	Non participants V=54 (%)	Total material N=7341 (%)
Age of mother		
≤20	14	16
21–35	77	69
>35	9	15
Social class		
upper	26	21
middle	38	34
lower	36	45
One parent family	13	12
Mother gainfully employed	35	35
full time	24	15
part time	11	20
Child in day nursery	9	6

Table 5 *Non participants earlier health supervision at Child Health Center*

	Number
Regular health supervision	31
Visited CHC only during first year of life	8
Has never visited CHC	15
Total	54

4 handicapped and cared for appropriately 7 private check up at 4 years ? language difficulties 1 against on principle 1 no information

neglected in the sens of inadequacy of health care provided for them but they seem to represent a small minority among the already small group of non participants It also appeared somewhat reassuring that more than half of the children had some other form of health supervision at 4 years of age There was documented evidence that not more than 4 children out of a total of 2877 had never been seen at a CHC nor had had health supervision at the age of 4 years (Table 5)

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For another 168 (2 3%) participation was refused by the parents for various reasons The hypothesis that these children constitute a group with special need of health supervision cannot be ruled out *a priori* (1) We therefore conducted a special study of non participants born in 1966 by collecting additional information from existing health records and by scrutinising the lists of patients currently treated at the Departments of Pediatrics Child Psychiatry Pediatric Surgery Orthopedics and Audiology and at the rehabilitation centres for motor and mentally handicapped children The parents of all those children whose records did not contain a satisfactory explanation for their non participation were interviewed by a trained pediatric nurse not involved in the activities of the Child Health Centres Of the 59 non participants 5 were found to have had the check up at a later date

Table 3 summarizes the main reasons for non participation in the remaining 54 cases Lack of interest or time were noted most often and for the majority of these children some other health supervision was provided The differences in background data (social class age marital status and gainful employment of mother arrangements for day care) between non participants and the total material were small and there was no evidence in favour of the hypothesis that non participants form a socially deprived risk group (Table 4) A few individual cases such as immigrants whose parents did not speak Swedish and those for whom no information could be obtained are probably to be looked upon as neglected children

Table 4 *Background data of 54 non participants*

	Non participants N=54 (%)	Total material N=7341 (%)
Age of mother		
≤20	14	16
21–35	77	69
>35	9	15
Social class		
upper	26	21
middle	38	34
lower	36	45
One parent family	13	12
Mother gainfully employed	35	35
full time	24	15
part time	11	20
Child in day nursery	9	6

Table 5 *Non participants earlier health supervision at Child Health Center*

	Number
Regular health supervision	31
Visited CHC only during first year of life	8
Has never visited CHC	15
Total	54

4 handicapped and cared for appropriately 7 private check up at 4 years 2 language difficulties 1 against on principle 1 no information

jected in the sense of inadequacy of health care pro- ed for them but they seem to represent a small minor among the already small group of non participants It appeared somewhat reassuring that more than half of children had some other form of health supervision at ears of age There was documented evidence that not re than 4 children out of a total of 2 877 had never been n at a CHC nor had had health supervision at the age of ears (Table 5)

COMMENTS

uantitative data concerning the results tained with this screening program will be esented in subsequent papers However a w issues must be discussed in the present ontext

Feasibility

he experience gained during the first 3 years as shown that the implementation of a ystematic screening program into a pre xisting health service is possible Data could e collected in a systematic way for every hild concerned the procedure was accepted y the population (high participation rate) and he staff and the increase in the number of ermanent staff necessary to compensate for he extra work load was acceptable (not more han 10%) Special examinations and treat nent could be offered to all children needing hem

Significance of program changes

Since routine health care rarely offers the op portunity for an experimental design the deci sion for changes in the program may not only be prompted by quantitative evidence—as in the case of the interview and the test of motor performance (see page 797)—but also by defi nite opinion of the primary staff (e g simplifi cation of data collection procedure) or of di rectly involved specialists (new test for speech development) and by other sources as well (literature etc) Changes likely to bring about an improvement cannot be postponed until all the planned quantitative evaluations have been performed Such intervening changes may therefore represent a source of variation in any analysis of the total material In the ensuing publications attention will be paid to the possible effects of these changes

3 *The decentralized approach*

The main advantages of the approach advo cated by the Swedish National Board of Health and Welfare are the preservation of the continuity of care the high degree of accessi bility and the improvement in the professional competence of the staff—especially the nurses —performing the examinations On the other hand the large number of persons involved implies a substantial risk of suboptimal or even definitely poor performance This aspect of inter observer variability will be dealt with in paper III (6)

4 *Theoretical framework for the subsequent analysis*

In order to decide whether the advantages of the decentralized approach really outweigh the drawbacks it remains to be demonstrated that the overall performance of the program is ac ceptable The ultimate goal of the program obviously is to bring about an improvement of the health status of children through early de tection and treatment of those pathologic con ditions and developmental anomalies which are amenable to treatment (9) An attempt to demonstrate the program s effectiveness in achieving this goal would only be successful if a follow up study could show that the state of health of a cohort of schoolchildren who at the age of 4 years were given a chance to partici pate in the screening and to undergo the necessary treatments is better than the state of health of a group of control children Such a study is in progress in Uppsala but conclusive results will first be available in a couple of years In the meantime however some partial aspects of effectiveness should be analysed because such preliminary analyses may already point to areas which certainly need to be improved now Two important parameters of effectiveness are the sensitivity and the specificity of the screening process In order to compute quantitative estimates of these

parameters additional criteria are needed which definitely separate the sick from the healthy individuals For many health problems of 4 year olds such objective criteria do not exist and therefore it would not have helped very much if a sample of the population had been re examined with more sophisticated methods in close connection with the screening A definite answer to the question whether the screening procedure has correctly identified most of the children with real problems can thus only be expected from the follow up study mentioned above It is possible however to define at least crude estimates of the sensitivity and the specificity on the basis of available data The specificity or rather the lack of specificity is reflected by the rate of unnecessary referrals This aspect will be treated in some detail in paper III (6) The best estimate of sensitivity which can at present be derived from the available data is a comparison of the total prevalence of previously known and newly detected health problems in our population with that found in centres offering an optimal chance that existing problems are detected (every child being examined by the same specially trained team) Paper II (5) will be devoted mainly to this purpose

REFERENCES

1. Kohler L Health control of four year-old children An epidemiological study of child health *Acta Paediatr Scand* Suppl 235 1973
2. North A F Research issues in child health A Head Start research seminar I Introduction and overview V An afterview *Pediatrics 45* 690 884 1970
3. Socialstyrelsen *Allman halsokontroll av fyraaringar* Socialstyrelsen redovisar Stockholm 1968
4. Sundelin C Parents experiences of child health centers A study in the urban district of Uppsala *Scand J Soc Med 3* 113 1973
5. Sundelin C & Vuille J C Health screening of four year olds in a Swedish county (II) Effectiveness in detecting health problems To be published
6. Sundelin C & Vuille J C Health screening of four year olds in a Swedish county (III) Variation of effectiveness among examining teams To be published
7. Vuillle J C & Groth T Anwendungsmoglichkeiten des Computers in der Padiatrie Praventivmedizinische Datenbank uber samtliche Kinder *Arithmed International 2* 33 1969
8. Vuille J C Den allmanna halsokontrollen av fyraåringar *Lakartidningen 68* 449 1971
9. World Health Organization Regional Office for Europe *Evaluation of MCH services in certain countries of the European region* Report of a Working Group Copenhagen 1973

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HEALTH SCREENING OF FOUR YEAR OLDS IN A SWEDISH COUNTY

II Effectiveness in Detecting Health Problems

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ABSTRACT Sundelin C and Vuille J C (Department of Paediatrics University Hospital Uppsala Sweden and the Institute of Social and Preventive Medicine University of Berne Berne Switzerland) Health screening of four year-olds in a Swedish county II Effectiveness in detecting health problems Acta Paediatr Scand 64 801 1975.—Quantitative results obtained during the first 3 years of operation of a routine health screening program for 4-year-olds are presented From these figures it is concluded that the *effectiveness* in detecting previously unknown health problems needing treatment is high enough to justify the continuation of this systematic health screening by the ordinary child health staff However the total prevalence of newly detected anomalies was lower in our population than in similar studies where the screening was performed by a few teams of specially trained professionals The consequences of this observation are briefly discussed

KEY WORDS Pre school children health screening effectiveness sensitivity referrals

Health screening of preschool children may either be performed by specialized screening centres (11) or it may be incorporated into the traditional system of health care provision The latter model implemented in the County of Uppsala since 1969 and later on in many other Swedish counties has been described with respect to organization methods and participation in a previous publication (17)

This article presents an attempt to estimate the sensitivity of the health screening which is one aspect of program effectiveness as defined in paper I (17) No results based on rescreening are still available neither from specialized screening centres nor from the decentralized routine model Thus the only possibility to day to assess the sensitivity is to compare the rates of previously known and newly detected health problems with those found in studies where optimal techniques could be used in well controlled experimental settings

METHODS

For organization screening methods coding and data processing system see paper I (17) For the *statistical analysis* the type (vision hearing speech mental emotional neurological physical) and degree (slight moderate severe) of any health problem were coded by the authors using the information gathered at the primary examination and the data submitted by the specialists Thereby a slight problem was defined as a condition for which treatment was recommendable but not absolutely necessary a moderate problem was a condition for which treatment was deemed necessary in order to avoid future impairment or suffering and a severe problem was any manifest handicap Case records form hospital departments were also consulted concerning those children whose parents had stated in the questionnaire that their child had had previous treatment for a chronic condition

A previously known health problem was thus defined as any chronic disease impairment or other anomaly which the parents knew about and which had been adequately treated before the check up Any similar pathological condition which was first detected in connection with the screening at 4 years was defined as *a newly detected health problem* if and only if the child was referred to a specialist and the specialist verified not only the existence of an abnormality but also the need for treatment In 13% of all referred cases no information was obtained from the

Table 1 *Rates of referrals per 100 examined children*

Specialist	1969 $n=992$	1970 $n=2\ 818$	1971 $n=3\ 863$	Whole period $n=7\ 173$
Psychologist	0 6	0 6	1 6	1 1
Psychologist & physician	4 8	3 1	3 4	3 5
Pediatrician	5 5	4 5	4 7	4 7
Pediatric surgeon	2 8	3 1	2 7	2 9
Orthopedic surgeon	1 2	2 8	2 3	2 4
Oto rhino laryngologist	1 5	2 2	2 7	2 3
Audiologist	5 1	2 3	4 2	3 6
Phoniatrist	0 9	1 0	7 0	3 8
Ophthalmologist	5 8	8 7	15 4	11 4
Plastic surgeon & dermatologist	1 2	1 1	1 2	1 2
Total	29 4	29 3	45 2	36 9

specialists Thus the tabulated rates of newly detected health problems represent a minimum estimate of the total benefit brought about by the program The category of previously known health problems includes all severely handicapped children who did not undergo the check up but who were registered at rehabilitation centres

RESULTS

Crude results concerning the rate of referrals and the prevalence of health problems (except dental problems) observed during the period 1969–70 will be presented Table 1 shows the rates of *referrals* to specialist departments for the three years of the study separately With three notable exceptions (ophthalmologist phoniatrist and audiologist) which will be discussed on pages 803–804 there were only minor fluctuations between the three years

Previously known newly detected and total prevalence of health problems

The rates of previously known and newly detected health problems as well as the total prevalence in the material from the whole period are shown in Table 2 The following observations emanating from the table are noteworthy

1 *The total prevalence is highest for physical problems* followed by eye anomalies emotional difficulties and hearing and speech impairments

2 In general severe problems are the least frequent minor anomalies occurring most often Exceptions from this general rule are visual defects the majority of which were classified as moderate and mental and neurological impairments where all three categories occur in approximately equal proportions

3 The relative importance of the screening program for early recognition of treatable health problems can be estimated by the ratio *newly detected problems/total prevalence* This ratio decreases with increasing degrees of severity However speech and emotional problems do not follow this rule probably be

Table 2 *Previously known newly detected and total prevalence of health problems Rates per 100 children*

	Slight			Moderate			Severe			Total		
	Prev known	Newly det	Total prevalence	Prev known	Newly det	Total prevalence	Prev known	Newly det	Total prevalence	Prev known	Newly det	Total prevalence
Vision	0 35	1 73	2 08	2 06	2 50	4 56	0 05	0 03	0 08	2 46	4 26	6 72
Hearing	0 14	0 99	1 13	0 10	0 28	0 38	0 10	0 00	0 10	0 34	1 27	1 61
Speech	0 17	1 06	1 23	0 11	0 43	0 54	0 00	0 01	0 01	0 28	1 50	1 78
Mental	0 07	0 29	0 36	0 08	0 14	0 22	0 27	0 01	0 29	0 42	0 44	0 87
Emotional	0 26	1 56	1 83	0 18	0 99	1 17	0 01	0 07	0 08	0 45	2 62	3 08
Neurological	0 14	0 07	0 21	0 13	0 03	0 15	0 28	0 03	0 31	0 55	0 13	0 67
Physical	3 76	3 62	7 39	1 37	0 78	2 15	0 24	0 08	0 32	5 37	4 48	9 86
Total	4 89	9 32	14 22	4 03	5 15	9 16	0 95	0 23	1 19	9 87	14 70	4 59

Including severely handicapped children who did not undergo the screening but who were registered at rehabilitation centres

Table 3 *Prevalence of health problems in present material compared with rates found in two similar studies*

	Lund (1)	Basle (?)	Present study (3)
Number of children examined	2 447	1 436	7 173
Per cent of target population	95 1	?	97 7
Examining team			
specialized	+	+	–
polyvalent	–	–	+
Rates per 100 children			
Visual defects	15 4	8 0	6 7 (8 0)
Hearing loss	3 9	0 1	1 6 (1 6)
Speech disturbance	–	3 3	1 8 (2 9)
Mental retardation	–	–	0 9 (1 0)
Emotional problems	–	3 5	3 1 (3 ?)
Neurological disturbances	3 3	–	0 7 (0 8)
Physical problems	1? 0 (4)	–	9 9 (9 5)

(1) See reference (11)
( ) See reference (?1)
(3) Figures in parentheses indicate the rates found in 1971 after several changes in screening program (17)
(4) Including 6 6% functionally unimportant orthopedic anomalies

ause there are no effective case finding mechanisms for this type of problem before the age of 4 years In all 60% of the health problems present among 4 year olds were first detected by the screening procedure Of the severe health problems 19% were identified in the same way

DISCUSSION

For reasons given in the introduction a comparison will be made with figures published from two screening centres using specialized staff (11 20) The comparison must be based on the total prevalence since the ratio of newly detected to previously known impairments depends largely on the effectiveness of earlier case finding mechanisms which may vary considerably between different places Nonetheless a comparison with figures published from other centres is not always easy because often only the crude numbers of anomalies suspected after the primary screening are given or overall referral rates without the rates of verification of the suspected anomalies by specialists (1 5 7) A further difficulty arises from the frequent lack of subdivision into categories of different severity and from disparities in the underlying classification

The findings from the three studies are presented in Table 3 Prevalence rates of similar magnitude were found in a prospective study of children 0–5 years of age in Newcastle upon Tyne (14) and for 7 year olds in the National Child Development Study (5)

Vision

As seen from Table 1 the referral rates for visual problems increased considerably from 1969 to 1971 This was due to a deliberate continuous review of the technique for visual screening used by the nurses and to special training sessions given by the pediatric ophthalmologist During the year 1971 the newly introduced automatic monitoring system (17) identified a number of children who had not been referred by the examining team though they satisfied the criteria for referral As a consequence of higher referral rates the total prevalence of known visual defects rose also and reached 8 0/100 in 1971 This is still substantially lower than the exceedingly high figure found by Kohler & Stigmar (12) In our material a further 5% of all children were found to need further ophthalmological observation since no definite diagnosis could be established by the ophthalmologist when the child was only 4 years old This may explain part of the difference between the two materials

Hearing

The decrease in referral rates to the audiologist from 1969 to 1970 was probably due to a raise in the pass level in audiometry and to more systematic retesting before referral The reason for the new increase in 1971 is not known there was no corresponding increase

in verified hearing defects since the suspected hearing loss could not be verified in the majority of the referred children There is no obvious explanation for the differences between the three studies in Table 3 with respect to the prevalence of hearing loss This question cannot be settled until figures from follow up examinations of the same populations become available

Speech

The seven fold increase in referrals between 1970 and 1971 (Table 1) was above all due to the introduction of a systematic test administered by auxiliary personnel (17) This increase in referrals was accompanied by a substantial increase in verified speech anomalies At 4 years of age development of normal speech is still going on fairly rapidly The prevalence of minor speech abnormalities is therefore highly dependent on the age at which the final diagnosis is made In Uppsala the specialist examination was postponed 1 to 1 ½ years after the screening in order to permit an observation of the spontaneous development The final prevalence in our material is similar to that found in Basle However much higher figures have been published from other studies (2 5), which illustrates the difficulties encountered in any attempt to define objective and generally acceptable criteria for speech anomalies

Physical problems

The effectiveness of diagnosing physical anomalies appeared quite satisfactory even when compared with the results from specialized centres All cases of *recurrent urinary tract infection* are included in this figure The total prevalence was 0 7/100 but the majority of these children had aldready been treated, and previously unrecognized bacteriuria was present in only 0 1/100 This figure is difficult to reconcile with the corresponding figure of 0 8/100 among girls found by Kohler Fritz & Schersten (10) It is possible that part of this discrepancy is due to the difficulties which many parents may have experienced in following the written instruction for collection of the urine sample

Neurological anomalies

The high rate of neurological anomalies found by Kohler is surprising, since several studies in Sweden have shown the prevalence of motor handicap and convulsive disease to be definitely lower than 1/100 (3 8 13 19) This inconsistency may well be explained by the inclusion in Kohler s material of many clumsy children who were given a final diagnosis of minimal brain dysfunction, whereas in Uppsala such children were either classified in the group of behavioural problems or—if no psychological problems were present—were assessed only in the case of an obvious handicap

Emotional problems

The true prevalence of psychological problems that require treatment is certainly higher than the 3 1% found in our study (2 4 9 16) and the 3 5% found in Basle A special analysis of our material has shown that a considerable number of children were not referred even though problems of importance were identified at the screening examinations of 4 year olds (15) Thus there is a strong suspicion that the sensitivity of our screening procedure was not satisfactory with respect to emotional problems An analysis of possibilities for improvement will be published in a separate report

Mental retardation

As could be expected the majority of severely retarded children were known and taken care of before the age of four years (Table 2) It can be anticipated that a significant proportion of the individuals with slightly or moderately reduced intellectual capacity will be able to manage an independent life quite successfully without special support (6) Therefore screening for mental retardation among preschool children must not aim at a precise assessment

of the intelligence quotient with the purpose to label all the individuals with subnormal performance in psychological tests The procedure should rather try to identify specifically those children in whom early educational intervention may exert a beneficial influence on their future development or those who present serious problems to their families Only long term observation of the total cohort of children will be able to show to what extent our screening procedure has been able to achieve this goal

GENERAL CONCLUSIONS

The majority of the newly detected health problems are probably of minor importance for the future development of the child On the other hand more than half of all moderate health problems were not known about before the check up and in view of the generally assumed high quality of preventive Pediatrics in Sweden it was rather surprising to find that among every 1000 four year olds there were still 2–3 children with previously undetected or not adequately treated disabling conditions essentially within the categories emotional and physical problems For these and for the moderately severe cases the screening was certainly important On the other hand the total numbers of identified health problems were lower in our study than the corresponding numbers from one specialized screening centre This is probably due to different definitions of the concept health problem and to an incomplete identification in our study of the less severe cases which are more difficult to distinguish from the normal children if no so phisticated methods are used This apparent lack of sensitivity is not serious enough to recommend the discontinuation of systematic health screening of 4 year olds by the ordinary child health staff but it points to the necessity of a continuous evaluation of achievements and a further analysis of factors associated with possible failures (18) The present study has already shown that the sensitivity in different respects can be improved through continuous education of personnel (e g vision screening) the use of simple tests in the hands of auxiliary staff (speech) and computerized monitoring of the compliance with certain referral criteria

REFERENCES

1 Belleville M & Green P B *Preschool multiphasic screening programs in rural Kansas Am J Public Health 62* 795 1971

2 Bengtsson I Bernler G Carlgren G Fallstrom S P Karlberg P Korsan Bengtsen M Larsen G Liedholm M Mortenson K Nachemson A von Sydow G & Torell P Goteborgsförsoket med halsokontroll av fyraåringar *Lakartidningen 69* 1893 1972

3 Brandberg O & Rundberg B Chronically disturbed children *Acta Paediatr Scand 51* 41 1962

4 Chamberlain R W Management of preschool behaviour problems *Pediatr Clin North Am 21* 33 1974

5 Davie R *Summary of the First Report of the National Child Development Study* King Thorne & Stace Ltd Hove 1967

6 Granat K & Granat S Below average intelligence and mental retardation *Am J Ment Defic 78* 27 1973

7 Heym H Modellstudie uber Vorsorge im Kleinkindesalter *Oeff Gesundheitswes 31* 470 1969

8 Holmdahl K Frekvensen av handikapp i barnaåldern och några synpunkter på deras omhandertagande *Opuscula Medica 15* 383 1970

9 Klackenberg G A prospective longitudinal study of children Data on psychic health and development up to 8 years of age *Acta Paediatr Scand* Suppl 224 1971

10 Kohler L Fritz H & Scherstén B Health control of four year-old children A study of bacteriuria *Acta Paediatr Scand 61* 289 1972

11 Kohler L Health control of four year old children An epidemiological study of child health *Acta Paediatr Scand* Suppl 235 1973

1 Kohler L & Stigmar G Vision screening of four year-old children *Acta Paediatr Scand 62* 17 1973

13 Lagergren J Motor handicapped children A study from a Swedish county *Dev Med Child Neurol 12* 56 1970

14 Miller F J W Court S D M Walten W S & Know E G *Growing up in Newcastle upon Tyne* (0–5 years) Oxford University Press Oxford 1960

15 Nilsson C *Den allmanna halsokontrollen av fyraaringar En utvardering av det psykologiska undersokningsprogrammet* Dissertation Uppsala 1973

16 Socialstyrelsen *Allman halsokontroll av fyraåringar* Socialstyrelsen redovisar Stockholm 1968

17 Sundelin C & Vuille J-C Health screening of four year-olds in a Swedish county I Organization methods and participation *Acta Paediatr Scand 64* 795 1975

18 Sundelin C & Vuille J C Health screening of four year olds in a Swedish county III Variation of effectiveness among examining teams To be published
19 von Sydow G *Children with physical handicaps in a Swedish county* The Swedish National Board of Health Stockholm 1967
20 Urbanczik A Erste Ergebnisse und Erfahrungen mit Vorsorgeuntersuchungen bei Kindergartenkindern (4 bis 5 jährigen) in Basel *Ther Umsch 30* 142 1973

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BREAST FEEDING AND BIOLOGICAL PROPERTIES OF FAECAL *E COLI* STRAINS

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ABSTRACT Gothefors L Olling S and Winberg J (Departments of Paediatrics and Clinical Bacteriology University of Umeå Umeå Sweden and Department of Immunology Institute of Medical Microbiology University of Goteborg Goteborg Sweden) Breast feeding and biological properties of faecal *E coli* strains Acta Paediatr Scand 64 807 1975.—Human milk may contribute to protection against gram negative infection by promoting intraluminal agglutination or killing of bacteria or by preventing bacterial attachment to epithelial surfaces This paper explores the effect of human milk on the sensitivity of faecal *E coli* to serum bactericidal activity and agglutination specificity factors which have been regarded as *related to virulence* Faecal *E coli* isolated from breast fed infants differed from those from formula-fed infants in two respects They were more sensitive to the bactericidal effect of human serum and more often spontaneously agglutinating *E coli* strains isolated from sources outside the gastro-intestinal tract that is the prepuce and female peri urethral region were in breast fed babies less sensitive than faecal strains The findings are compatible with the hypothesis that a breast milk factor favours the proliferation of mutant strains The observed effects of breast milk might be associated with decreased bacterial virulence and be one of the ways in which breast feeding protects against infection

KEY WORDS Breast feeding *E coli* virulence bactericidal effect of normal serum intestinal colonization

Gastro intestinal infections are less common in breast fed than in formula fed infants (cf 7 11) This seems to be due not only to increased contamination during formula feeding but also to a protective effect of human milk (18) Recently Larguia et al (13) showed that as little as 5 ml colostrum/kg b w /24 hr to prematures was suffficient to prevent enteropathogenic *E coli* (EPEC) and salmonella gastro intestinal infections Still more interesting was their observation that EPEC and salmonella occurred in the faeces of some of the infants without causing any symptoms Human milk may also reduce the risk of septicaemia and meningitis of the newborn infant (29)

The way in which human milk contributes to the protection against clinical infection is not fully understood The main reason why the number of faecal *E coli* and other gram negative bacilli in breast fed infants is notably smaller than in formula fed babies (28) is probably the low buffering capacity and high lactose content of breast milk (4) Other properties of human milk may have a more marginal effect which however can become important when virulent bacteria have colonized the newborn infant Intraluminal agglutination (26) or killing (1) of potential pathogens prevention of bacterial attachment to the epithelial surface (5) or reduction in number (19) may then play a role Neutralization of bacterial virulence factors may be another way in which breast milk can provide protection a possibility hitherto unexplored In this paper faecal *E coli* strains

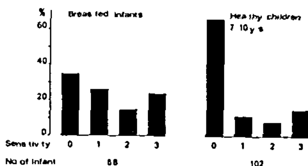


Fig 1 Bactericidal effect of human serum on faecal *E coli* strains isolated from 68 breast fed infants and from 102 school children (0=strains highly resistant 3=strains highly sensitive see Methods)

from breast fed and from formula fed infants were compared with respect to resistance to the bactericidal effect of normal human serum and their tendency to agglutinate spontaneously These two properties might be related to the virulence of the bacteria (12 22)

MATERIAL AND METHODS

The material consisted of 91 full term healthy infants delivered in hospital They stayed there during the first week of life and were then sent home The infants were all from Umeå The control material consisted of 102 school children living in Goteborg (14 20) The term breastmilk or human milk refers only to the native product not processed in any respect The formula used was fat and protein adapted with low salt content

Faecal *E coli* were cultured either from rectal swabs or from stool specimens Samples from the peri urethral region were collected as follows The prepuces of the males were irrigated with 0 05 M phosphate buffer In the girls a gelatin block was pressed gently against the peri urethral region A slice about 2–3 mm thick of the block was cut off and dissolved in 0 05 M phosphate buffer

The fluid used for irrigation of the prepuces the phosphate buffer dissolved gelatin slices and the faecal swabs were seeded on Conradi Drigalski agar

A simplified *E coli* O grouping was performed as earlier described (15) Three sets of polyvalent antisera to 68 different O antigen groups were used Cultures non specifically agglutinated by all sera were referred to as spontaneously agglutinating Such strains frequently formed rough colonies on the agar plate Strains that were not agglutinated by the available sera and that did not agglutinate spontaneously were referred to as not agglutinable

The bactericidal test has been reported earlier (20) The bacterial suspension to be tested was mixed with normal human serum and incubated at 37 C for 30 minutes Brain–Heart–Infusion broth was then added to inhibit further bactericidal activity and to obtain optimal growth conditions The suspension was incubated at 37 C for 3–5 hrs The percentage of surviving bacteria was determined by densitometric comparison with three standard tubes without serum in which the test organism had been diluted to 50% 10% and 1% of the suspension used in the test The results of the analyses were rated as follows 0 denotes <50% of the bacteria killed 1 denotes 51–90% killed 2 denotes 91–99% killed and 3 denotes >99–100% killed *In some experiments complete lysis* was denoted by 4

RESULTS

A *Serum bactericidal sensitivity of faecal E coli in school children and in breast fed new born infants*

The bactericidal effect of normal human serum on strains isolated from faeces of 68 breast fed infants 3–7 days old, was investigated From one faecal culture in each infant six colonies were randomly chosen and examined Of the 408 strains investigated 400 were *E coli* The median value for bactericidal sensitivity (in whole number) was calculated for the *E coli* strains from each patient The results were compared with those found *in 102 healthy* children 7–10 years old Only one strain from each of these patients was examined (20) The results obtained in the two groups are given in Fig 1 The groups were compared by χ^2 test and the strains isolated from breast fed infants were found to be more sensitive to the bactericidal effect than those from school children (χ =16 86 3 d f $p<0$ 001) Similar *results were obtained when comparison was* made with *E coli* strains chosen at random from 232 healthy school children (14)

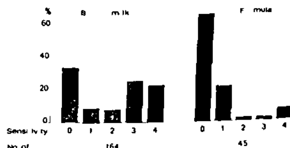


Fig 2 Bactericidal effect of human serum on strains isolated from faeces of breast fed and artificially fed infants 0–2 months old (0=strains *highly resistant* 4=strains highly sensitive see Methods)

Table 1 Bactericidal effect of serum on E coli isolated from faeces and prepuce or peri urethral region in breast fed infants

	Number of infants investigated	Comparison of bactericidal sensitivity*			p
		No differences between F and U†	F more sensitive than U	F less sensitive than U	
Boys	35	12	18	5	<0 025
Girls	21	5	1[illegible]	4	<0 025

The comparison was made between the mean of six strains from each habitat.
† F=strains from faeces U=strains from prepuce or peri urethral region
Wilcoxon s test for paired observations

B *Serum bactericidal sensitivity of faecal E coli in breast fed and in formula fed infants*

The difference in A might be explained by several factors such as food age or environment We therefore tried to reduce the possible influence of the two latter factors by comparing the sensitivity of *E coli* strains isolated from infants who were fed on either formula or human milk Twelve infants were examined four times each—first in the hospital nursery and then at 3 5 and 9 weeks after birth when living at home Six random colonies were chosen from each faecal specimen Only *E coli* was examined for bactericidal sensitivity One infant was on formula all the time 4 were fed human milk all the time and 7 were first given breast milk but later formula The infants were allocated to the breast milk group as long as at least one meal consisted of human milk This might have reduced the extent of existing differences between breast fed and formula fed infants

The results are shown in Fig 2 and suggest an increased sensitivity of the strains isolated from breast fed infants than of those isolated from the formula fed infants Since several infants appeared in both feeding groups statistical calculation of the difference did not seem justified

It may be of interest that out of the 7 infants who switched from breast feeding to formula feeding in 3 of them the change was associated with a shift of the aerobic gram negative flora from mainly *E coli* to mainly *Klebsiella* or *Citrobacter*

C *Serum bactericidal sensitivity of faecal praeputial and female peri urethral E coli flora*

If the increased sensitivity of *E coli* strains isolated from breast fed infants was due to the continuous influence of a faecal factor such as IgA copro-antibodies one would expect gram negative bacteria outside the gastro-intestinal tract to be less sensitive The male prepuce is one habitat with an abundance of gram negative bacteria (16) Smaller amounts are found in the female peri urethral region (2)

The peri urethral and faecal flora of 35 boys and 21 girls all breast fed and less than one week old was examined Six random colonies from each faecal and peri-urethral specimen were selected and the *E coli* strains were examined The mean bactericidal sensitivity of each sample was calculated and the difference for each pair of faecal-prepuce flora and faecal-vulvar flora was calculated with Wilcoxon s test for paired observations (Table 1) The strains isolated from the faecal flora were more sensitive than those isolated from the prepuce ($p<0\ 025$) Also in the females the faecal strains were more sensitive than those isolated from the peri urethral region ($p<0\ 025$)

D *Spontaneous agglutination of faecal E coli in breast fed and formula fed infants*

The occurrence of spontaneously agglutinating *E coli* in faeces from breast fed and

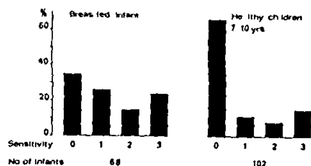


Fig 1 Bactericidal effect of human serum on faecal *E coli* strains isolated from 68 breast fed infants and from 102 school children (0=strains highly resistant 3=strains highly sensitive see Methods)

from breast fed and from formula fed infants were compared with respect to resistance to the bactericidal effect of normal human serum and their tendency to agglutinate spontaneously These two properties might be related to the virulence of the bacteria (12 22)

MATERIAL AND METHODS

The material consisted of 91 full term healthy infants delivered in hospital They stayed there during the first week of life and were then sent home The infants were all from Umeå The control material consisted of 102 school children living in Goteborg (14 20) The term breastmilk or human milk refers only to the native product not processed in any respect The formula used was fat and protein adapted with low salt content

Faecal *E coli* were cultured either from rectal swabs or from stool specimens Samples from the peri urethral region were collected as follows The prepuces of the males were irrigated with 0 05 M phosphate buffer In the girls a gelatin block was pressed gently against the peri urethral region A slice about 2–3 mm thick of the block was cut off and dissolved in 0 05 M phosphate buffer

The fluid used for irrigation of the prepuces the phosphate buffer dissolved gelatin slices and the faecal swabs were seeded on Conradi Drigalski agar

A simplified *E coli* O grouping was performed as earlier described (15) Three sets of polyvalent antisera to 68 different O antigen groups were used Cultures non specifically agglutinated by all sera were referred to as spontaneously agglutinating Such strains frequently formed rough colonies on the agar plate Strains that were not agglutinated by the available sera and that did not agglutinate spontaneously were referred to as not agglutinable

The bactericidal test has been reported earlier (20) The bacterial suspension to be tested was mixed with normal human serum and incubated at 37 C for 30 minutes Brain–Heart–Infusion broth was then added to inhibit further bactericidal activity and to obtain optimal growth conditions The suspension was incubated at 37 C for 3–5 hrs The percentage of surviving bacteria was determined by densitometric comparison with three standard tubes without serum in which the test organism had been diluted to 50% 10% and 1% of the suspension used in the test The results of the analyses were rated as follows 0 denotes <50% of the bacteria killed 1 denotes 51–90% killed 2 denotes 91–99% killed and 3 denotes >99–100% killed In some experiments complete lysis was denoted by 4

RESULTS

A Serum bactericidal sensitivity of faecal E coli in school children and in breast fed new born infants

The bactericidal effect of normal human serum on strains isolated from faeces of 68 breast fed infants 3–7 days old, was investigated From one faecal culture in each infant, six colonies were randomly chosen and examined Of the 408 strains investigated 400 were *E coli* The median value for bactericidal sensitivity (in whole number) was calculated for the *E coli* strains from each patient The results were compared with those found in 102 healthy children 7–10 years old Only one strain from each of these patients was examined (20) The results obtained in the two groups are given in Fig 1 The groups were compared by χ^2 test and the strains isolated from breast fed infants were found to be more sensitive to the bactericidal effect than those from school children ($\chi^2=16$ 86 3 d f $p<0$ 001) Similar results were obtained when comparison was made with *E coli* strains chosen at random from 232 healthy school children (14)

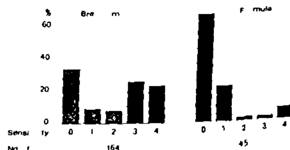


Fig 2 Bactericidal effect of human serum on strains isolated from faeces of breast fed and artificially fed infants 0–2 months old (0=strains highly resistant 4=strains highly sensitive see Methods)

Table 1 *Bactericidal effect of serum on E coli isolated from faeces and prepuce or peri urethral region in breast fed infants*

	Number of infants investigated	Comparison of bactericidal sensitivity[a]			
		No differences between F and U[b]	F more sensitive than U	F less sensitive than U	p
Boys	35	12	18	5	<0 025
Girls	21	5	12	4	<0 025

[a] The comparison was made between the mean of six strains from each habitat
[b] F strains from faeces U=strains from prepuce or peri urethral region
Wilcoxon s test for paired observations

B *Serum bactericidal sensitivity of faecal E coli in breast fed and in formula fed infants*

The difference in A might be explained by several factors such as food age or environment We therefore tried to reduce the possible influence of the two latter factors by comparing the sensitivity of *E coli* strains isolated from infants who were fed on either formula or human milk Twelve infants were examined four times each—first in the hospital nursery and then at 3 5 and 9 weeks after birth when living at home Six random colonies were chosen from each faecal specimen Only *E coli* was examined for bactericidal sensitivity One infant was on formula all the time 4 were fed human milk all the time and 7 were first given breast milk but later formula The infants were allocated to the breast milk group as long as at least one meal consisted of human milk This might have reduced the extent of existing differences between breast fed and formula fed infants

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D *Spontaneous agglutination of faecal E coli in breast fed and formula fed infants*

The occurrence of spontaneously agglutinating *E coli* in faeces from breast fed and

Table 2 *Occurrence of spontaneously agglutinating faecal E coli in breast fed and formula fed infants*

	Breast fed		Formula fed	
	No of strains	%	No of strains	%
Groupable[a]	193	70	83	59
Not agglutinable[b]	51	19	53	38
Spontaneously agglutinating	30	11	4	3
	274	100	140	100

[a] With one of 68 specific *E coli* antisera
[b] Not agglutinable with the 68 antisera

formula fed infants (definitions, see above) was investigated in 34 infants (one faecal specimen from each of 11 infants and two–four from each of 23 infants) Samples were taken during the first week and at 3 5 and 9 weeks after delivery Six colonies were chosen at random from each sample and the *E coli* clones were analysed The results are shown in Table 2 The analysis suggested that the frequency of spontaneously agglutinating strains usually rough colony formers was higher in breast fed than in formula fed infants The two groups were not compared statistically because some infants occurred in both groups

DISCUSSION

The study showed that faecal *E coli* isolated from breast fed infants differ in two respects from those isolated from formula fed infants They are more sensitive to the bactericidal effect of human serum and they are more often spontaneously agglutinating *E coli* from the formula fed infants resembled those from school children in sensitivity to serum It was also shown that *E coli* colonizing the prepuce and female peri urethral region of breast fed infants were less sensitive than corresponding faecal strains

The cause of the difference in serum bactericidal sensitivity (SBS) in breast fed and in formula fed infants is not clear The sensitivity of a strain to bactericidal activity probably depends on the surface and structure of the cell wall The cell envelope of *E coli* consists of two membranous structures outer and inner membrane, respectively (21) The outer membrane has been shown to constitute a barrier to several bactericidal compounds This barrier is partly dependent on the lipopolysaccharide moiety of this outer membrane A loss of carbohydrates is known to be associated with an increased permeability of the outer membrane (8, 25 27) The O antigen deficient R mutants are thus more sensitive to the serum bactericidal activity than strains with complete O antigen (S forms) (22) The R forms also have a tendency to agglutinate spontaneously

The most probable explanation for the observed difference between faecal strains is that a breast milk factor exerts a selective pressure in the gut and thereby favours strains with high SBS and tendency to spontaneous agglutination The high resistance observed in preputial and peri urethral strains indicates that once outside the reach of the selection pressure in the gastro intestinal tract the strains with a complete polysaccharide part of the lipopolysaccharide will again thrive well

The way in which breast milk favours R mutants as well as strains with high SBS is uncertain In experimental cholera in germ free mice the appearance of homologous antibodies was very often associated with a serotypic conversion and with the emergence of rough strains (24) This course of events could be prevented by immunosuppression This might be an experimental parallel to the colonization of the newborn with *E coli* The influence of the antibodies on bacterial properties in the mice might in in the infants have been exerted by IgA antibodies in breast milk

Specific antibodies may bind to bacteria in the gastro intestinal tract (3) and prevent bacterial attachment to epithelial cells (5) Since lipopolysaccharide deficient bacteria are probably less efficient as antigens the

IgA-coated S forms will be out grown by R mutants presumably without attached antibodies In long standing asymptomatic urinary infections in school children isolated strains often have a high SBS (20) or are R mutants (10) In fact the successive transition during long term observations from *smooth to rough strains and from resistant to* sensitive strains have been observed (17) It has been suggested that this represents an adaptation to the host s defence which enables the strains to survive (9) This might be a parallel to our observations on faecal *E coli* from breast fed infants

More data are required if the presence of IgA antibodies in breast milk is to be used as an argument for breast feeding (6) Such information is badly needed but cumulative data show that breast milk really does something to the host s defence This might be of importance especially in colonization with virulent strains

ACKNOWLEDGEMENTS

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REFERENCES

1 Adinolphi M Glynn A A Lindsay M & Milne C M *Serological properties of gamma A antibodies to Escherichia coli* present in human colostrum *Immunology 10* 517 1966

2 Bollgren I & Winberg J The periurethral aerobic *bacterial flora in healthy boys and girls* In preparation

3 Brandtzaeg P Fjellanger I & Gjeruldsen S T Adsorption of immunoglobulin A onto oral bacteria in vivo *J Bacteriol 96* 47 1968

4 Bullen C L & Willis A T Resistance of the breast fed infant to gastroenteritis *Br Med J III* 338 1971

5 Fubara E S & Freter R Protection against enteric bacterial infection by secretory IgA antibodies *J Immunol 111* 395 1973

6 Gellis S S *Year book of Paediatrics* Chicago 1974 p 18

7 Goldman A S & Smith C W J Host resistance factors in human milk *Pediatrics 82* 1082 1973

8 Gustafsson P Nordstrom K & Normark S Outer penetration barrier of *Escherichia coli* K 12 Kinetics of the uptake of gentian violet in wild type and envelope mutants *J Bacteriol 116* 893 1973

9 Hanson L Å Host-parasite relationships in urinary tract infections *J Infect Dis 127* 726 1973

10 Hanson L Å Jodal U Lincoln K & Lindberg U Is asymptomatic bacteriuria a sign of serious disease? *Br Med J I* 530 1972

11 Hanson L Å & Winberg J Breast milk and defence against infection in the newborn *Arch Dis Child 47* 845 1972

12 Henkel W & Commichau R Serumresistenz und Nephropathogenität antigenetisch definierter Stamme von *E coli Med Microbiol Immunol 156* 297 1971

13 Larguia A M Urman J Ceriani J M O Donell A Stoliar O Martinez J C Buscaglia J C Wells S Quiroga A & Irazu M Immunidad local en el recién nacido Primera experiencia con la administracion de calostro homano a recién nacidos pretermino *Arch Argentinos Pediatr 72* 109 1974

14 Lidin Janson G Unpublished

15 Lincoln K Lidin Janson G & Winberg J Resistant urinary infections resulting from changes in resistance pattern of faecal flora induced by sulphonamide and hospital environment *Br Med J III* 305 1970

16 Lincoln K & Winberg J Studies of urinary tract infections in infancy and childhood II *Acta Pediatr Scand 53* 307 1964

17 Lindberg U Hanson L Å Jodal U Lidin Janson G Lincoln K & Olling S Asymptomatic bacteriuria in schoolgirls II Differences in *Escherichia coli* causing asymptomatic and symptomatic bacteriuria *Acta Pediatr Scand* In Press

18 Mata L J & Urrutia J J Intestinal colonization of breast fed children in a rural area of low socioeconomic level *Ann N Y Acad Sci 176* 93 1971

19 Michael J G Ringenback R & Hottenstein S The antimicrobial activity of human colostral antibody in the newborn *J Infect Dis 124* 445 1971

20 Olling S Hanson L Å Holmgren J Jodal U Lincoln K & Lindberg U The bactericidal effect of normal human serum on *E coli* strains from normals and from patients with urinary tract infections *Infection 1* 24 1973

21 de Petris S Ultrastructure of the cell wall of *Escherichia coli* and the chemical nature of its constituent layers *J Ultrastruct Res 19* 45 1967

22 Rowley D *The virulence of strains of Bacterium coli* for mice *Br J Exp Pathol 35* 5 8 1954

23 Rowley D Sensitivity of rough gram negative bacteria to the bactericidal action of serum *J Bacteriol 95* 1647 1968

24 Sack R B & Miller C Progressive changes of vibrio serotypes in germfree mice infected with *Vibrio cholerae J Bacteriol 99* 688 1969

25 Schlect S & Westphal O Antibiotica Empfindlichkeit bei S und R formen von *Salmonella minnesota Naturwissenschaften 10* 494 1968

26 Shearman D J C Parkın D M & McClelland D B L The demonstration and function of antibodies in the gastrointestinal tract *Gut 13* 483 1972
27 Tamaki S Sato T & Matsuhasi M J Role of lipopolysaccharides in antibiotic resistance and bacteriophage adsorption of *Escherichia coli* K 12 *J Bacteriol 105* 968 1971
28 Tissier H Repartition des microbes dans l intestin du nourisson *Ann Inst Pasteur 19* 109 1905
29 Winberg J & Wessner G Does breast milk protect against septicaemia in the newborn? *Lancet I* 1091 1971

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RESPIRATORY INSUFFICIENCY SYNDROME (RIS) IN PRETERM INFANTS WITH GESTATIONAL AGE OF 32 WEEKS AND LESS

Neonatal Management and Follow up Study

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ABSTRACT Carlsson J and Svenningsen N W (Department of Paediatrics University Hospital Lund Sweden) Respiratory insufficiency syndrome (RIS) in preterm infants with gestational age of 32 weeks and less Neonatal management and follow up study Acta Paediatr Scand 64 813 1975 —The clinical entity of respiratory insufficiency syndrome (RIS) i e irregular breathing leading to recurrent apnea and bradycardia in an otherwise healthy preterm infant has been studied in respect of symptomathology and management with intensive case including ventilatory support During a 4-year period 26 of 103 infants with gestational age ≤32 weeks and mean birth weight 1 304 g (range 710 to 1 830 g) developed RIS In most infants the initial apnea occurred after 2 and before 72 hours post delivery but in some infants later Because of progressive hypoxemia and acidosis 15 of the 26 RIS infants required IPPV treatment The 76% survival rate of RIS infants seems to justify intensive care with ventilatory support even in the smallest preterm infants with RIS especially as the follow up study performed at 15 months to 3½ years of age showed neurological sequelae in only 3 of 20 surviving babies i e 15% sequelae rate

KEY WORDS Preterm infants Respiratory Insufficiency Syndrome (RIS) apnea neonatal intensive care Intermittent Positive Pressure Ventilation (IPPV)

For many years it has been observed that periodic irregular breathing is common in otherwise healthy preterm infants It is also well known that some of these infants breath ing periodically tend to develop apneic spells

In 1967 Raiha (20) classified a group of highly premature infants as having Respiratory In sufficiency Syndrome (RIS) These prema tures had no respiratory symptoms until 48–72 hours after birth when they became apneic and developed respiratory failure without signs of any other pathologic condition The same syndrome has by other authors been called Recurrent Neonatal Apnea of Prematurity (18) the premature baby who forgets to breathe (21) or preterm apnea (23)

The purpose of this paper is to study the effects of intensive care including ventilatory support of infants with RIS

MATERIAL AND METHODS

During a 4-year period 1970–73 103 low birth weight (LBW) infants with a gestational age of 32 weeks or less were admitted to the Neonatal Unit of the Children s Clinic at the University Hospital of Lund Their birth data were plotted on Swedish standard curves for the relationship between birth weight and gestational age (22) The degree of maturity was also evaluated by assessment of external features neurological development and head circumfer ence (7)

Twenty six of the 103 infants fulfilled the *criteria of RIS* i e *having a gestational age of 32 weeks or less and with irregular breathing lead ng to recurrent apnea several hours after birth and with normal chest X ray* Apnea was

Table 1 *Initial ventilator settings during Intermittent Positive Pressure Ventilation (IPPV) of Respiratory Insufficiency Syndrome (RIS) infants and procedures at weaning from Intermittent Positive Pressure Ventilation (IPPV) with Continuous Positive Airway Pressure (CPAP)*

Initial ventilator settings	
Respiration rate	25–30 per minute
Peak inspiration pressure	10–15 cm H_2O
Positive End Expiratory Pressure (PEEP)	0–2 cm H_2O
Inspiration/expiration ratio	1 2
Gasflow and volume	
Loosco Amsterdam Ventilator	constant tidal volume setting
Servo 900 Ventilator	constant flow volume controlled with triggering
Supplemental O_2 concentration	none or 25–30% O_2
Weaning from IPPV	
CPAP via endotracheal tube	2–4 cm H_2O in 5–10 minute periods *per hour intermittently for 4 to* 8 hours
Extubation followed directly by	
CPAP via face chamber	2–4 cm H_2O for 24 to 48 hours or longer periods when needed Pressure is lowered to 2 cm H_2O for *at least 8 hours before final weaning* from the continuous positive pressure
Supplemental O_2	25–30% O_2 only if required during IPPV treatment

defined as a period of non breathing during which cyanosis and slowing of the heart rate below 100 beats per minute occurred *Irregular breathing* was defined as ventilatory pattern consisting of a series of breaths interspersed with 3–15 second long apnoic episodes without bradycardia (8)

Another 9 infants with gestational age of 32 weeks or less also had apnoic attacks but did not fulfil the criteria of RIS (vide supra) for the following reasons 7 infants with gestational age between 25 and 27 weeks and birth weights between 650 and 1050 g were apnoic from birth or developed apnea within 30 to 60 min thereafter Six of these died within 12 hours of age with autopsy revealing intracranial intraventricular hemorrhage in 5 infants and total pulmonary atelectasis in one infant one infant with asphyxia was intubated at birth and treated with mechanical ventilation but died from septicaemia at 10 days of age and autopsy showed intracranial hemorrhage as well Of the remaining 2 infants with gestational age of 30 weeks one infant with birth weight 1360 g intubated and ventilated immediately after birth died at 6 hours of age from tentorial rupture with intracranial hemorrhage the other infant with birth weight 1630 g had normal chest X ray but developed *idiopathic respiratory distress syndrome* with apnea requiring mechanical ventilation and died at 5 days of age with autopsy findings of hyaline membranes and intracranial *hemorrhage These infants were not included in the RIS* groups

All infants were nursed in incubators with a high environmental humidity (60–80%) The skin temperature was maintained at *35 5°C through servo-control* Oxygen was administered if necessary via an oxygen hood in concentrations required to keep Pa_{O_2} above 50 mmHg Breathing was supervised continuously by a nurse the supervision was facilitated by use of an apnea alarm mattress (Vickers Medical Engineering UK) or impedance respimeter (SAAB Medical Engineering Linkoping Sweden) An umbilical artery catheter was inserted on admission and arterial blood samples were taken 3 to 6-hourly for analyses of acid–base status and blood–gas levels A small air column placed in the catheter allowed registration of pulse rate without opening incubator doors and thus without intervening in incubator temperature humidity or oxygen concentration during registration Repeated chest roentgenograms were taken to exclude *congenital malformations* or changes in the pulmonary parenchyma

Intravenous glucose solutions were given through the umbilical vein from 2–4 hours after birth Total or supplemental parenteral nutrition[1] was initiated from 12–24 hours after birth Peroral feeding was gradually introduced during the first days

A single occasional apnea was treated *by peripheral* stimulation or by ventilation with mask and bag If repeated attacks of apnea of increasing duration and during these attacks simultaneous bradycardia below 100 beats per minute occurred endotracheal intubation was performed and intermittent positive pressure ventilation (IPPV) initiated The respirators used were Loosco Amsterdam Respirator *(Loos & Co Amsterdam Holland) and Servo Ventilator* 900 (Siemens Elema AB Solna Sweden) A CPAP face chamber (CPAP FC 100 Siemens Elema AB Solna Sweden (1) was used for weaning the infants off the respirator treatment (Table 1)

Follow-up study

Before discharge ophthalmologic examination sono-encephalogram transillumination of the skull lumbar puncture with cytologic examination of the cerebrospinal fluid (26) EEG ECG pulmonary X ray pulmonary function testing (2) and neurological evaluation were performed

After discharge the infants were controlled every second month in the outpatient clinic where *neurological and developmental* evaluation transillumination of the skull sono-encephalogram pulmonary X ray and in IPPV infants pulmonary function tests were repeated

The Student s *t* test was used for assessment of significance of difference in group comparisons

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[1] Intermittently administered aminoacids (Vamin glucose) for 3½ hours and fat (20% Intralipid AB Vitrum Stockholm Sweden) for ½ hour

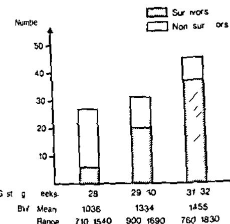


Fig 1 Number of survivors and birth weight in the total material of infants with gestational age of 32 weeks and less (BW = birth weight in grams)

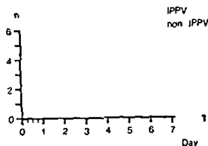


Fig 3 26 RIS infants Day of initial apnea (*n* = number of infants)

RESULTS

Total material

Survivors and non survivors with respective gestational ages are presented in Fig 1 As expected the survival rate increases with increasing gestational age and birth weight The survivors (63 infants) had a mean birth weight of 1 393 g (range 710–1 830) and a mean gestational age of 30 6 weeks (range 27–32) The non survivors (40 infants) had a mean birth weight of 1 164 g (range 710–1 830) and a mean gestational age of 28 0 weeks (range 25–32) IPPV treatment was given to 14 of the 63 surviving infants and to 15 of the 40 non survivors

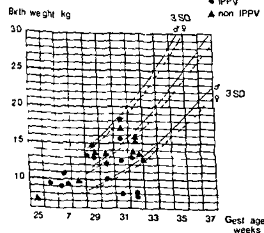


Fig 2 Birth weight in relation to gestational age in 6 RIS infants

RIS infants

The birth weight in relation to gestational age of the 26 RIS infants is presented in Fig 2 The mean birth weight was 1 218 g (range 710–1 830) Twenty three of the infants were appropriate for gestational age and 3 were small for gestational age As seen in Fig 3 most infants got their initial apnea after 2 hours and before 72 hours of life However in some infants the initial apnea appeared after 72 hours of age

Table 2 shows relevant clinical data of these infants The number of survivors in the non IPPV treated and the IPPV treated group was 9 out of 11 and 11 out of 15 respectively The gestational age and the Apgar scores were similar in both groups whereas the birth weight was slightly lower in the IPPV group ($p < 0.05$)

The mean age at initial apnea was the same in both groups and so were also the oxygen concentration in the incubator the incubator temperature and the infant rectal temperature

In the IPPV group ventilator treatment was started on average after 52 hours (range 1–168 hours) of irregular breathing because of the occurrence of 2 or more recurrent apnea with falling heart rate during the apneic attack in spite of peripheral (cutaneous) stimulation The mean duration of IPPV treatment was 8 2

Table 2 *Clinical data of 26 infants with respiratory insufficiency syndrome (RIS)*

				Initial apnea							Follow up examinations		
					Environmental							Abnormalities	
Case no Sex	Birth weight (g)	Gest age (w)	Apgar score (1–10 min)	Age (days)	O_2%	temp (°C)	Rectal temp (°C)	IPPV duration (days)	Additional complications at age (days)	CSF cytology abnormal	Age (mo)	Pulmonary	Neurological
Non IPPV													
Survivors													
1 ♂ (twin 2)	1 270	33	10–10	5	30	33 5	36 3	–	–	0	22	None	None
2 ♀	1 360	29	10–10	6	25	34	36 2	–	Sepsis (8)	0	15	None	Slight spastic diplegia with athetosis
3 ♀	1 700	31	6–7	1	25	32	36 2	–	–	–	24	None	None
4 ♀ (twin 1)	1 340	32	8–10	2	–	32	36 8	–	Sepsis (4)	0	20	Pneumonia×1	None
5 ♂	1 520	32	10–9	13	–	31 5	36 4	–	–	0	15	Bronchitis×1	None
6 ♀ (twin 2)	1 580	32	6–10	1	60	33	37 0	–	IRDS (2)	+	18	None	None
7 ♂ (twin 2)	1 350	30	7–10	2	50	32	36 5	–	–	–	26	None	None
8 ♂	1 370	32	4–8	2	40	34	35 8	–	–	+	17	None	None
9 ♂	1 470	29	8–10	4	30	33 5	36 5	–	Necrotizing enterocolitis (14)	+	18	None	None
Non survivors													
10 ♀ (twin 1)	970	27	9–9	1	40	36	36	–	Sepsis (10) died day 11 *Autopsy* HM pulmonary atelectasis hemorrhagic diathesis				
11 ♀	710	25	7–10	1	30	30	29 6	–	Melena (2) died day 2 *Autopsy* Pulmonary atelectasis				
Mean	1 321	30 2	7 7–9 3	3 4	33 6	33 1	35 8	–					
IPPV													
Survivors													
12 ♂	1 080	27	–	7	30	36	36 2	7	–	–	42	None	None
13 ♀ (twin 1)	1 300	32	10–10	4	30	31	36	2	Bil/s 31 mg% (4)	+	24	Recurrent bronchitis	None
14 ♂	910	27	2–2	1	50	32	35 5	21	–	0	18	Recurrent bronchitis	None
15 ♂	1 330	29	8–8	4	30	32 5	37	17	–	0	18	Bronchitis×1	None
16 ♀	1 260	31	8–10	3	36	36	36 8	11	Bil/s 16 mg% (4)	0	18	Bronchitis×1	None
17 ♀	1 560	31	10–10	2	30	3? 5	36	6	Sepsis (5)	0	24	None	Hypsarrythmia at 3 mo of age Severe psychomotor retardation spastic diplegia
18 ♀	760	32	1–4	2	–	37 5	35 7	4	–	0	24	Bronchi	None

19 ♀	8 0	3	4–8	3	30	35	36 5	5	Sep (28)	+	18	Bron h t s×2	Sl ght pa t d pl gia otherw e no n al
20 ♂	1 100	29	5–9	1	35	32	36	9	Sep s (2)	0	24	Recu re t b onchit s	None
21 ♂ (triple I)	1 010	30	9–10	7	35	34	36 6	8	–	0	18	None	None
22 ♂ (triple II)	1 220	10	8–10	7	35	31	36 8	7	–	0	18	None	None
Non sur ivors													
23 ♀	930	27	9 10	4	43	37	36	3	Died day 8 *Autopsy* HM pneumoniae				
24 ♀	800	31	7–9	2	40	33	36 8	1½	Died day 8 *Autopsy* Incipient HM				
25 ♀	940	26	?–5	1	40	37	35	14	Sepsis (13) died day 14 *Autopsy* Pneumoniae encephalomalacia				
26 ♀	1 830	31	9–10	5	24	31	36 8	2	Aspiration and died day 14 *Autopsy* Pneumoniae				
Mean	1 137	29 6	6 5–8 2	3 5	33 4	33 8	36 2	8 2					
p value	<0 05	n s	n s	n s	n s	n s	n s	–					

53 75 876

days (range 36 hours to 21 days) followed by a weaning off period in the CPAP face chamber for usually 24 to 48 hours (range 12 hours to 8 days)

Additional postnatal complications appeared in 4 of 9 surviving infants in the non IPPV group and in 5 of 11 surviving infants in the IPPV group as shown in Table 2 Hypoglycemia or hypocalcemia during apnea was not observed

Clinical data including autopsy findings of the non surviving 6 RIS infants (Nos 10 11 23 24 25 26) are also shown in Table 2 Five of them had a birth weight less than 1 000 g and 4 had a gestational age below 28 weeks The sixth infant had a birth weight of 1 830 g and was accidentally lost in an aspiration pneumonia on day 14

Table 3 shows the number of peripheral stimulations required because of apnea during irregular breathing in the IPPV (before treatment) and the non IPPV group respectively More intensive stimulation was required in the IPPV group wheras the non IPPV infants responded instantaneously to peripheral stimulation

Fig 4 shows the mean and standard error of mean (S E M) values of pH Pa_{O_2} Pa_{CO_2} and base deficit (BD) before and during periods of irregular breathing with two or more attacks of apnea There is a fall in pH and Pa_{O_2} and a rise in base deficit in the group of infants later requiring IPPV therapy On the contrary there is an increase of pH and Pa_{O_2} in the non IPPV group

Follow up examinations

These are summarised in Table 2 In the non IPPV group 3 of 7 and in the IPPV group 2 of 10 surviving infants showed CSF cytological signs of intracranial hemorrhage Skull transillumination sonoencephalogram and ophtalmological examinations were normal in all babies EEG performed at discharge from the neonatal unit were normal in all but one infant (No 14) At reexamination at 6 months of age the EEG had normalized Head circumference (related to gestational age at birth) was within normal range at latest follow up examination in all

Table 3 *26 Respiratory Insufficiency Syndrome (RIS) infants Number of peripheral stimulations required during irregular breathing in the Intermittent Positive Pressure Ventilation (IPPV) and non Intermittent Positive Pressure Ventilation (non IPPV) group*

No peripheral stimulations	Number of infants	
	IPPV group	Non IPPV group
1	–	1
2–5	–	–
6–10	1	3
11–50	7	3
51	7	4

[a] Before IPPV start

babies (Fig 5) Neurological examination performed at 15 to 42 months of age was abnormal in 3 infants (Nos 2 17 19) as shown in Table 2 Recurrent bronchitis requiring hospitalization in later infancy was encountered in 3 babies (Nos 13 14 20) Pulmonary X ray showed moderate bronchopulmonary dysplasia in all 3 infants but had normalized at the latest follow up examination after 18 months of age in all but one (No 20)

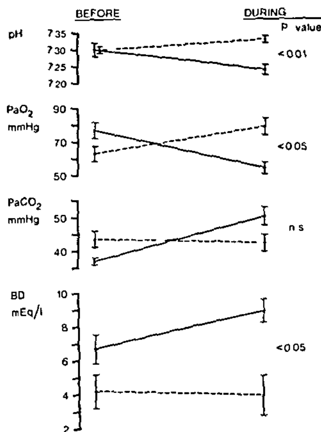


Fig 4 Means and standard error of mean (S E M) values of arterial pH Pa_{O_2} Pa_{CO_2} and base deficit (BD) in 26 RIS infants (IPPV group — non IPPV group) before and during irregular breathing interrupted by apnea (n s =not significant)

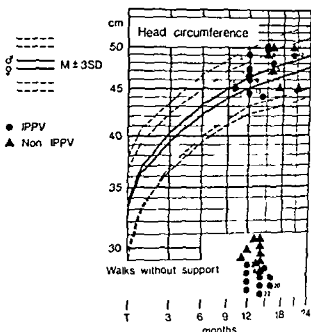


Fig 5 Head circumference at follow up examination and time of walking without support Correction has been made for gestational age at birth (*T*=term i e 40 gestational weeks) Numbers – case numbers

DISCUSSION

Definition

Episodes of irregular breathing occur frequently in preterm infants especially during the first days of life The incidence of irregular (periodic) breathing was found by Fenner et al (6) to be 94 5% in infants weighing less than 2 500 g at birth Occasionally these episodes lead to apnea A number of pathological conditions in the newborn period may cause apnea e g intracranial hemorrhage pulmonary disease septicemia and hypoglycemia (4) The additional postnatal complications in our material (Table 2) were not considered as etiologic to apnea as they did not appear concomitantly with RIS but usually several days later

A diversity of names has been given to the

occurrence of recurrent apnea in the preterm infant not related to postnatal complications We use the name Respiratory Insufficiency Syndrome—RIS—to mean irregular breathing leading to recurrent apnea with bradycardia in an otherwise healthy preterm infant We have seen this clinical entity only in infants of 32 weeks gestation or less

In the present material of infants with a gestational age ≤32 weeks 25% developed RIS The same incidence was found by Miller et al (15) studying a material of infants with birth weight between 1 001 and 1 750 g

The rapid onset of hypoxia and acidosis following bradycardia (8 17 19 25) seen in some RIS infants implies serious danger of cerebral damage (9) with development of i a spastic diplegia (14) This threat justifies early and intensive treatment of the RIS infant in order to avoid such sequelae Different therapeutic regimens have been recommended e g increase of ambient oxygen concentration (15) and administration of theophyllamin (13) Controlled respiration as an early preventive measure as adopted in the present investigation was originally proposed by Reid & Mitchell (18)

Clinical features

In the small preterm infant developing RIS irregular breathing with occasional apnea usually starts during the first 72 hours of life (20) However as shown in the present study in several infants signs of RIS do not commence until after 72 hours (Fig 3)

It has been stated that apnea is more frequent at high environmental temperatures (4) This could not be confirmed in the present study Although the infants requiring IPPV had more frequent and severe apnea than the infants of the non IPPV group no difference was observed in environmental temperature conditions (Table 2)

In infants not requiring IPPV treatment peripheral (cutaneous) stimulation was sufficient to restore respiration immediately consequently no acid–base or blood–gas abnormalities developed In contrast the infants with recurrent apnea requiring IPPV treatment showed a gradually declining arterial oxygen tension and acidosis in spite of increased oxygen concentration in the incubator in some cases to 80% O_2 before starting IPPV (Fig 4) Similar observations were made by Rigatto et al (19) Furthermore an increase of ambient O_2 did not reduce the number or duration of subsequent apnea in the IPPV group On the other hand in the non IPPV group an increase of ambient O_2 resulted in a rise of the arterial oxygen tension and a lower frequency of apnea It seems to us that our findings are indicative of a progressive respiratory insufficiency in those RIS infants who need IPPV treatment for survival Studies of pulmonary mechanics in RIS infants are thus of importance in order to further elucidate the pathophysiology of this clinical entity (2)

Outcome

The survival rate of very low birth weight infants has improved considerably after introduction of modern methods of care (3 16 23 27) In our material of 26 RIS infants 20 survived (76%) Fifteen infants required IPPV treatment and 11 of these (73%) survived (it should be noted that the mean gestational age of the IPPV treated RIS infants was 29 6 weeks and the mean birth weight 1 147 g) We consider that our results strongly support the opinion of those authors (18 24) advocating IPPV treatment in infants with the clinical entity of severe RIS Supportive therapy including supplemental parenteral nutrition has possibly also been of importance for the final outcome (10) However as longterm sequelae must be taken into consideration in order to justify intensive care of these very small infants follow up studies have been performed with regard to pulmonary and neurological sequelae in the 20 surviving RIS infants In the present material 3 of 11 infants treated with IPPV in the neonatal period have suffered from recurrent bronchitis related to moderate bron

chopulmonary dysplasia After 10 months of age the clinical condition and pulmonary function tests have improved considerably in all 3 infants (2) At 18 months of age only one of these 3 infants (No 20) still has abnormal pulmonary X ray findings These findings are in agreement with those of Johnson et al (12) who found that those infants with bronchopulmonary dysplasia who survived beyond 7 months of age had no cardiopulmonary disability when they reached 5 years of age

Cerebrospinal fluid cytological signs of intracranial hemorrhage were found in 5 of 17 examined survivors in the postneonatal period before discharge from our neonatal unit However neurological and developmental abnormalities were observed in only 3 of 20 surviving RIS infants i e 15% at the latest follow up examination performed between 15 months and 3½ years of age (Table 2) In other words 17 of 20 RIS infants or 85% were indistinguishable from normal children at that age Of course it cannot be excluded that minor cerebral dysfunctions might be detected at a later age Consequently continued follow up studies are in progress At the present time our results are similar to those reported by other neonatal units after intensive care of very low birth weight infants showing an incidence of neurological handicaps varying between 9 5 and 25% (3 5 16 23 27) Still it must be realized that comparisons between different materials are difficult as many variables often differ, e g indications for ventilatory support way of nutrition, incidence of infections and so on In the previous reports (vide supra) either all infants treated with ventilatory support or all infants below a certain birth weight usually 1 500 g have been considered *in toto* The prognosis of the clinical entity of RIS has been evaluated by itself only in the present study although it is pointed out in one study (23) that infants with preterm apnea carry a very good prognosis for life Also in agreement with our findings hypoxia (and acidosis) is considered the most important cause not only of mortality but also of cerebral handicaps (8 9 23, 25) Thus intensive care including early ventilatory support when required seems valuable in the treatment of RIS infants

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REFERENCES

1 Ahlstrom H Jonsson B & Svenningsen N W Continuous positive airway pressure with a face chamber in early treatment of idiopathic respiratory distress syndrome *Acta Paediatr Scand* 62 433 1973
2 Ahlstrom H Pulmonary mechanics in infants surviving severe neonatal respiratory insufficiency *Acta Paediatr Scand* 64 69 1975
3 Calame A & Prod hom L S Pronostic vital et qualite de survie des prematures pesant 1500 g et moins à la naissance soignés en 1966–1968 *Schweiz Med Wschr* 102 65 1972
4 Daily W J R Klaus M & Meyer H B P Apnea in premature infants monitoring incidence heart rate changes and an effect of environmental temperature *Pediatrics* 43 510 1969
5 Dinwiddie R Mellor D H Donaldson S H C Tunstall M E & Russell G Quality of survival after artificial ventilation of the newborn *Arch Dis Child* 49 703 1974
6 Fenner A Schalk U Hoenicke H Wendenburg A & Roehling T Periodic breathing in premature and neonatal babies incidence breathing pattern respiratory gas tensions response to change in the composition of ambient air *Pediatr Res* 7 174 1973
7 Finnstrom O Studies on maturity in newborn infants VI Comparison between different methods for maturity estimation *Acta Paediatr Scand* 61 33 1972
8 Girling D J Changes in heart rate blood pressure and pulse pressure during apnoic attacks in newborn babies *Arch Dis Child* 47 403 1972
9 Grunnet M L Culress R G Bray P F & Jung A L Brain changes in newborns from an intensive care unit *Develop Med Child Neurol* 16 320 1974
10 Heird W C Driscoll J M & Winters R W Total parenteral nutrition in infants of very low birth weight *In* Ciba Foundation Symposium ?7 (new series) *Size at birth* J A Churchill Ltd London 1974 p 329
11 Hornbein T F The puzzle of periodicity *Pediatrics* 50 183 1972
12 Johnson J D Malachowski N C Grobstein R Welsh D Daily W J R & Sunshine P Prognosis of children surviving with aid of mechanical ventilation in the newborn period *J Pediatr* 84 272 1974

13 Kouzemko J A & Paala J Apnoic attacks in the newborn treated with aminophyllin *Arch Dis Child* *48* 404 1973
14 McDonald A Cerebral palsy in children of very low birth weight *Arch Dis Child 38* 579 1963
15 Miller H C Behrle F C & Smull N W Severe apnea and irregular respiratory rythms among premature infants *Pediatrics 23* 676 1959
16 Rawlings G Reynolds E O R Stewart A & Strang L B Changing prognosis for infants of very low birth weight *Lancet I* 516 1971
17 Reid D H S & Tunstall M E Recurrent neonatal apnoea *Lancet II* 155 1965
18 Reid D H S & Mitchell R G Recurrent neonatal apnoea *Lancet I* 786 1966
19 Rigatto H & Brady J Periodic breathing and apnea in preterm infants I Evidence for hypoventilation possibly due to central respiratory depression *Pediatrics 50* 202 1972
20 Raiha N C R Experiences with neonatal intensive care in Finland *Ross Conference on Pediatric Research 59 64 1968*
21 Sinclair J C The premature baby who forgets to breathe *New Eng J Med 282* 508 1970
22 Sterky G Swedish standard curves for intrauterine growth *Pediatrics 46* 7 1970
23 Stewart A L & Reynolds E O R Improved prognosis for infants of very low birth weight *Pediatrics 54* 724 1974
24 Strang L B Clinical indications for mechanical ventilation in newborn infants *Biol Neonate 16* 142 1970
25 Svenningsen N W & Siesjo B K Cerebrospinal fluid lactate/pyruvate ratio in normal and asphyxiated neonates *Acta Paediatr Scand 61* 117 1972
26 Sornas R A new method for the cytological examination of the cerebrospinal fluid *J Neurol Neurosurg Psych 30* 568 1967
27 Vapaavouri E K & Raiha N C R Intensive care of small premature infants *Acta Paediatr Scand 59* 353 1970

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LDH ISOENZYMES IN CHILDREN

A Comparison of Values from Capillary and Venous Blood

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ABSTRACT Christoffersen J Marner, T and Raabo E (Department of Paediatrics Glostrup Hospital Glostrup Denmark) LDH isoenzymes in children A comparison of values from capillary and venous blood Acta Paediatr Scand 64 822, 1975 — A comparison has been made of serum values of LDH isoenzymes obtained by simultaneous sampling of capillary and venous blood in 40 normal children No satisfactory correlation between the paired values could be found Evaluation of the pattern of serum LDH isoenzymes in children therefore requires a reference i e normal values of isoenzymes obtained by the same technique of blood sampling

KEY WORDS LDH isoenzymes children capillary/venous blood

Lactate dehydrogenase (LDH) is an enzyme which is catalytic in the transformation of lactic acid to pyruvic acid The total LDH activity in serum is due to 5 isoenzymes which can be separated by electrophoresis The relative concentration of these isoenzymes varies from organ to organ In serum the isoenzyme pattern is determined by the contribution from various organs

The LDH isoenzymes in serum are of great importance in the diagnosis of heart liver and blood diseases particularly among adults In childhood the isoenzymes are valuable in the diagnosis of both muscular dystrophies (1 2) and Tay Sachs disease (6) Previously the measurement of serum LDH was based upon samples of venous blood Now capillary blood samples are used as well This study was carried out to clarify whether the LDH isoenzyme patterns in capillary and venous blood are identical in the individual child at any given moment

MATERIAL AND METHODS

The material consisted of 40 normal children aged 1 month to 8 years Capillary and venous blood samples were drawn simultaneously from each child The serum was separated within half an hour and both kinds of samples were analysed by the same technique The serum was applied on electrophoretic paper (cellulose acetate) and

Table 1 *Mean values and standard deviation of LDH isoenzymes in capillary and venous blood from 40 normal children expressed in per cent of total LDH*

Same method of determination in both kinds of sampling has been used

	LDH I	LDH II	LDH III	LDH IV	LDH V	Total LDH
Cap	25 3±8 5	31 5±4 5	21 8±4 8	11 1±4 2	10 2±3 9	54 5±12 1 U/l
Ven	29 3±8 5	31 8±4 0	20 7±4 6	9 8±3 5	8 6±3 6	50 6±11 4 U/l

Table 2 *LDH values from capillary and venous blood compared by means of Spearman rank correlation with t test*

p=probability (Student's t test) r=correlation coefficient (Spearman rank correlation test)

	LDH I	LDH II	LDH III	LDH IV	LDH V	Total LDH
p	0 001	0 005	0 001	0 001	0 001	0 001
r	0 7103	0 4?51	0 6178	0 488	0 4980	0 5856

the fractions were eluted in acetone and determined photometrically The technique is described in detail in a previous paper (4)

RESULTS

Table 1 represents mean values and standard deviations of LDH isoenzymes in 40 children expressed in per cent of the total LDH The standard deviations for each isoenzyme were of the same order of magnitude in capillary and venous samples As seen in Table 2 a monotonous co-variation between the capillary and venous values was found by Student's *t* test ($p<0\ 005$) However the Spearman rank correlation between the paired values was unsatisfactory ($r<0\ 7$) except in LDH I In LDH I only the capillary value may represent the venous one with a reasonable reliability and vice versa This is seen clearly in Figs 1 and 2

DISCUSSION

In this study a correlation between the paired values of LDH isoenzymes in capillary and venous blood was found being however insignificant for practical use Haemolysis leads to an alteration of the isoenzyme pattern in serum due to a high concentration of LDH I in erythrocytes (3) Capillary sampling is more likely to produce haemolysis than venous sampling which may explain the lack of satisfactory correlation Another possible explanation could be a contamination of capillary blood by isoenzymes from connective tissue which would counteract the expected direction of the correlation as LDH I is relatively low in this tissue (5) In spite of a varying degree of haemolysis and contamination in capillary sampling standard deviations of the

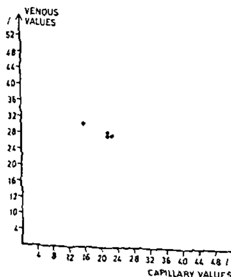


Fig 1 Venous and capillary values of LDH I expressed in per cent of total LDH

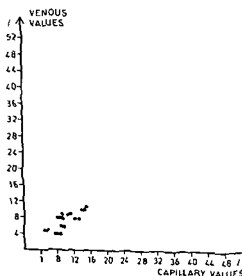


Fig 2 Venous and capillary values of LDH V expressed in per cent of total LDH

capillary samples were not found to exceed those of the venous samples The reliability of the two methods of sampling is therefore thought to be equally good The unsatisfactory correlation between the paired values in the individual child however indicates that evaluation of the pattern of serum LDH isoenzymes in children requires a reference, i e normal values obtained by the same technique of blood sampling

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REFERENCES

1 Farriaux J P Adam E & Fontaine G Les isoenzymes de la lactico-déshydrogenase en practique pédiatrique *Path Biol 17* 379 1969
2 Hooshmand H Dove J & Suter C The use o serum lactate dehydrogenase isoenzymes in th diagnosis of muscle diseases *Neurology 19* 26 196
3 Lehmann V Isoenzymes *Nord Med 86* 1041 197
4 Raabo E Colorimetric method for demonstratin lactic dehydrogenase isoenzymes separated by pape electrophoresis *Scand J Clin Lab Invest 15* 40 1963
5 Richterich R Schafroth P & Aebi H A study o lactic dehydrogenase isoenzyme pattern of human tis sues by adsorption elution of Sephadex DEAE *Cli Chem Acta 8* 178 1963
6 Saifer A Schneck L Perle Guta & Volk B W Lactate dehydrogenase isoenzyme distribution in th cerebral sphingolipidoses and other neurological dis orders *Neurology 19* 147 1969

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SURVIVAL AFTER MASSIVE PULMONARY HAEMORRHAGE IN THE NEONATAL PERIOD

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ABSTRACT Thomas D B (Research Unit for the Newborn Children s Medical Research Foundation Royal Alexandra Hospital for Children and The Women s Hospital Crown Street Sydney Australia) Survival after massive pulmonary haemorrhage in the neonatal period Acta Paediatr Scand 64 825 1975.—4 babies with massive pulmonary haemorrhage were seen in an 18 month period 2 had severe rhesus haemolytic disease and the other 2 severe respiratory problems There was evidence of a profound respiratory acidosis and hypoxia prior to the onset of the haemorrhage 2 babies survived the episode and subsequently developed normally Adequate ventilation and attendance to the treatment of blood coagulation disorders may have been a factor in their survival

KEY WORDS Massive pulmonary haemorrhage neonate blood coagulation disorders

Massive pulmonary haemorrhage is diagnosed when blood or blood stained fluid is seen in the upper airways There has been only one reported survivor (9) Suggested predisposing factors have included hypoxia (16 24) intra partum asphyxia (8 19) oxygen toxicity (3 21) rhesus haemolytic disease (2 7) coagulation disorders (15 20) infection (1 2 5 6 13) hypothermia (4 11 17 18) babies small for gestational age (12 13) and congenital heart disease (2) Recently it has been suggested (10) that it could result from acute left ventricular failure and haemorrhagic pulmonary oedema

Case reports and the results of some investigations are here reported in 4 infants with massive pulmonary haemorrhage 2 of whom survived Investigations are summarised after the case reports

CASE REPORTS

Case 1

A girl induced at 35 weeks gestation because of severe rhesus haemolytic disease Weight 041 g Apgar score 6 and 8 at one and 5 minutes Admission temperature 34 5°C Cord haemoglobin 6 6 g/100 ml and total bilirubin 7 5 mg/100 ml Cardiac failure was treated with digoxin and frusemide 100% oxygen was given for the first 18 hours of life Exchange transfusion at 3 hours resulted in marked clinical improvement A hypoglycaemic convulsion occurred at 36 hours At 92 hours 2 units of platelets were given because of the persistent thrombocytopaenia At 106 hours a second exchange transfusion was followed by another 2 units of platelets Progressive hypoxia and cardiac failure necessitated intermittent positive pressure respiration (IPPR) from 152 hours Chest X ray at this time showed collapse of the basal segments of the right lower lobe with opacities in both upper lung fields There had as yet been no signs of external bleeding At 156 hours she was accidentally disconnected from the ventilator and was hypoxic for 2–3 minutes Soon after 2–3 ml of fresh blood was aspirated from the endotracheal tube Plasma protein concentration 6 hours before the haemorrhage was 3 8 g/100 ml X ray shortly after the haemorrhage showed increased opacification in the right lung and this appearance persisted for a further 4 days A third exchange transfusion was performed at 160 hours with fresh heparinized blood followed by 4 units of platelets 24 hours later there was a further hypoglycaemic convulsion and more fresh blood was aspirated from the trachea She survived and at one year of age appeared physically and neurologically normal

Case 2

A girl with severe rhesus haemolytic disease An intra uterine transfusion was performed 2 weeks before an elective induction at 34 weeks gestation Apgar score 3 at one minute Weight 1870 g Cord haemoglobin 6 g/100 ml

Total bilirubin 4 5 mg/100 ml IPPR was started immediately after birth 200 ml of ascitic fluid were aspirated from the peritoneal cavity Following treatment of cardiac failure and an exchange transfusion her clinical condition improved She was extubated at 11 hours and nursed in 100% oxygen Chest X ray showed poorly expanded lungs but no specific pattern Prior to the 2nd exchange transfusion at 41 hours a small amount of bright blood was seen in her mouth and she appeared restless Bradycardia and cyanosis occurred after the start of the transfusion and she was re intubated and ventilated Soon after fresh blood was aspirated from the endotracheal tube and the bleeding persisted for 4 hours Plasma protein concentration was 4 8 g/100 ml 10 hours before the haemorrhage The haematocrit of the lung fluid was 33 when the corresponding arterial haematocrit was 41 Writhing movements developed towards the end of the transfusion and the X ray showed irregular opacities through the right lung and left upper lobe She died at 4 days Autopsy showed massive bilateral pulmonary haemorrhage and kernicterus There was no intraventricular haemorrhage

Case 3

A boy delivered spontaneously at 34 weeks gestation Weight 1700 g Apgar scores 3 and 5 Intubated at birth Severe hyaline membrane disease (HMD) Treated with continuous positive airways pressure from 4 to 98 hours Thereafter nursed in 40% oxygen At 103 hours he developed bradycardia and cyanosis but was not apnoeic The symptoms resolved spontaneously but recurred 5 hours later this time with apnoea which did not respond to bag and mask ventilation Gasping respirations followed and he required IPPR 2 hours later he became restless and blood stained fluid appeared in the endotracheal tube and persisted for 6 hours Plasma protein concentration at this time was 3 6 g/100 ml Following this he recovered uneventfully Chest X ray after the haemorrhage showed confluent opacities in the right lung and left upper lobe which reverted to normal in 2 days

Case 4

A boy delivered spontaneously at 27 weeks gestation Weight 900 g Apgar scores 6 and 8 He had mild HMD with progressive apnoea IPPR was commenced at 25 hours and 2 hours later blood stained fluid was seen in the endotracheal tube and this persisted for 12 hours Plasma protein concentration was 3 8 g/100 ml 9 hours before the haemorrhage Chest X ray after the haemorrhage showed a coarse reticular pattern through both lung fields which persisted up to the time of death at 79 hours Autopsy showed bilateral massive pulmonary haemorrhage some areas of pneumonia and a massive bilateral intraventricular haemorrhage

Acid–base and blood gas measurements

The progress of arterial pH Pa_{CO_2} acid–base and Pa_{O_2} in the period prior to the pulmonary haemorrhage are illustrated in Fig 1 Most striking were the signs of progressive respiratory acidosis with a falling pH and rising Pa_{CO_2} in all 4 babies Metabolic acidosis was present The 4 babies had Pa_{O_2} levels of 120 100 75 and 55 mmHg when last measured prior to the haemorrhage

Sodium intake and plasma electrolytes

Case 2 received 7 mEq of sodium bicarbonate initially and a further 7 mEq over the next 24 hours to correct a metabolic acidosis The other babies did not receive supplementary sodium and total oral intake did not exceed 3 mEq/kg/24 hours Electrolyte determinations were all normal except case 4 who had a sodium of 148 mEq/l at 40 hours rising to 153 mEq/l just before death at 79 hours

Investigation and treatment of coagulation disorders

Methodology has been described previously (23) Details of the tests performed are listed in Table 1 The factor concentrate PPSB (factors II VII IX & X) was used to correct deficiencies of the prothrombin complex usually when the thrombotest was below 20% Details of the treatment of coagulation disorders are indicated in Table 1

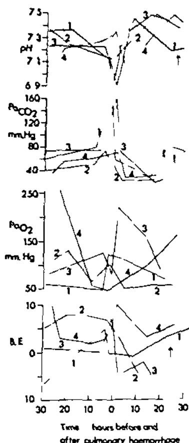


Fig 1 Serial measurements of pH Pa_{CO_2} base excess and Pa_{O_2} in the 4 babies before and after the pulmonary haemorrhage (Arrows indicate time of *second haemorrhage in case 1*)

Table 1 Serial coagulation tests in 4 babies who developed massive pulmonary haemorrhage

Hours of age	Haema tocrit	Fibrinogen (vols %)	FDP (µg/ml)	Thrombin time (sec)	PTTK (sec)	Thrombo-test (%)	Platelets (×10/mm)	Treatment
Normal		? 0–3 5	4	12–16	60–100	17–35	300–450	
Case 1								
3½	3?	1 1	3?0	40	82	13	60	Exchange transfusion
6	34	0 ?						
9		0 3				22		
31		0				16 5		
3?	35	? 0	40	65	82		65	PPSB 2 ml
55	36	0				8		
79	33	0	40			35	45	Platelets 2 units
10?	30	1 5	40	34	141	27	380	Exchange transfusion platelets 2 units
1 6	37	2 4	40	?3	107	33	55	
150	30	2 2	0	18	85		70	Exchange transfusion fresh heparinized blood platelets 4 units
174	34	8	10	13	87	31	2?0	
Case 2								
1½	?4	2 5	160	12	119	?6	500	Exchange transfusion
11	34	2 4	640	30	?3	25	360	
?2	45	3 0	160	9	95	?3	05	Exchange transfusion
53	45	3 0	80	13	7	23	130	PPSB ? ml
6?	46	3 9				?5	80	Exchange transfusion
80	52	4 2	?0	15	62	28	100	
Case 3								
12						13 5		PPSB 2 ml
130	37	3 6	?0	18	180	33	505	PPSB 2 ml at 1?1 hours
Case 4								
1		1 5	40			16		PPSB 2 ml
17	38	? 2				49		Fresh frozen plasma ?8 ml from 18 hours
41	0	3 3				33		
7?						25		

DISCUSSION

In all 4 babies there was evidence of a progressive respiratory acidosis up to the time of the pulmonary haemorrhage and in Case 1 this pattern was repeated prior to her 2nd haemorrhage In addition all the babies suffered an extra clinically obvious hypoxic insult just before they bled As suggested by Cole et al (10) the combination of hypoxia and acidosis could precipitate acute left ventricular failure and elevated left atrial and pulmonary pressures The plasma protein concentration determined around the time of the haemorrhage were all low This has been shown to potentiate pulmonary oedema (14)

The babies were all treated with IPPR prior to the haemorrhage and in no case did the pulmonary haemorrhage occur more than 4 hours after the commencement of ventilation It would seem likely that the factors which caused the haemorrhage were present at the time of intubation

Simmons et al (22) have demonstrated an association between hypernatraemia ad intraventricular haemorrhage and it is of interest that the baby who died with a massive intraventricular haemorrhage (case 4) was the

only baby who was hypernatraemic Radiological changes seen around the time of the *haemorrhage were varied and non specific*

Treatment was along conventional lines apart from the attempted correction of coagulation disorders *and this may have been a* reason for the survival of 2 babies, as it has been suggested (10) that coagulation disorders could *exacerbate or prolong a haemorrhage* once it had occurred

Shortly after the haemorrhage in Case 1, and exchange transfusion was performed with fresh heparinized blood, and 4 units of platelets were given The resultant improvement in coagulation status may explain why she survived the first haemorrhage and was able to cope with the 2nd 24 hours later Case 3 received 2 ml of PPSB at 12 hours of age No further tests were performed because of rapid *clinical improvement One hour before the* pulmonary haemorrhage a further 2 ml of PPSB was given because of rapid deterioration *in his condition When full testing was* performed 8 hours after the start of the haemorrhage only mild abnormalities were detected The 2 episodes of hypoxia prior to the haemorrhage may well have caused a reduction in the concentration of factors of the prothrombin complex and the PPSB may have been sufficient to maintain a relatively normal coagulation *state during the haemorrhage*

With reference to those who died in Case 2 no specific treatment for coagulation disorders was *given* until 11 hours after the haemorrhage The most obvious coagulation abnormality in this case was the progressive thrombocytopaenia This was not treated because of the signs of cerebral damage which were apparent shortly after *the haemorrhage* Case 4 was not adequately monitored and it was not possible to assess his coagulation status at the *time of the haemorrhage*

These findings would suggest that massive pulmonary haemorrhage does not necessarily carry the gloomy prognosis hitherto assigned to it Awareness of the effects of hypoxia on the circulation and coagulation mechanism together with prompt and adequate treatmen should help to reduce the mortality from pulmonary haemorrhage

REFERENCES

1 Ahvenainen E K Massive pulmonary haemorrhage *in the newborn Ann Paediatr Fenn 2* 44 1956
2 Ahvenainen E K & Call J D Pulmonary *hemorrhage in infants Am J Pathol 28* 1 1952
3 Boothby C B & de Sa D J Massive pulmonary haemorrhage *in the newborn A changing pattern Arch Dis Child 48* 21 1973
4 Bower B D Jones L F & Weeks M M Cold injury in the newborn a study of 70 cases *Br Med J I* 303 1960
5 Browne F J Pneumonia neonatorum *Br Med J I* 469 1922 *a*
6 Browne F J Neonatal death *Br Med J II* 590 1922 *b*
7 Chessells J M & Wigglesworth J S Haemostatic failure in babies with rhesus isoimmunisation *Arch Dis Child 46* 38 1971
8 Clifford S H The effects of asphyxia on the newborn infant *J Pediatr 18* 567 1941
9 Cole A P & Entress A Continuous positive air ways pressure in infants *Lancet I 505 1974*
10 Cole V A Normand I C S Reynolds E O R & Rivers R P A Pathogenesis *of hemorrhagic pulmo* nary edema and massive pulmonary hemorrhage in the *newborn Pediatrics 51 175 1973*
11 de Sa D J & Maclean B S An analysis of massive *pulmonary haemorrhage in the newborn* infant in Oxford 1948–68 *J Obstet Gynaecol Br Commonw 77* 158 1970
12 Esterly J R & Oppenheimer E H Massive pulmonary hemorrhage in the newborn *J Pediatr* 69 3 1966
13 Fedrick J & Butler N R Certain causes of neonatal death IV Massive pulmonary haemorrhage *Biol Neonate 18* 243 1970
14 Guyton A C & Lindsey A W Effect of elevated left atrial pressure and decreased plasma protein concentration on the development of pulmonary edema *Circ Res 1* 649 1959
15 *Hathaway W E Coagulation problems in the* newborn infant *Pediatr Clin North Am 17* 929 1970
16 McAdams *A J Pulmonary hemorrhage in the* newborn *Am J Dis Child 113* 255 1967
17 Mann T P & Elliot R I K Neonatal cold injury due to accidental exposure to cold *Lancet I* 229 1957
18 Pollen J C Matteo A L Petrucelli A L & Toledo N Pulmonary haemorrhage in the newborn infant *Ann Paediatr Fenn 3* 727 1957
19 Rowe S & Avery M E Massive pulmonary hemorrhage in the newborn *Clinical considerations J Pediatr 69* 12 1966
20 Roberts J T Davies *A J & Bloom* A L Coagulation studies in massive pulmonary haemorrhage of the newborn *J Clin Pathol 19* 334 1966

21 Shanklin D R & Wolfson S L Therapeutic oxygen as a possible cause of pulmonary hemorrhage in premature infants *N Engl J Med 277* 833 1967

22 Simmons M A Adcock E W Bard H & Battaglia F C Hypernatremia and intracranial hemorrhage in neonates *N Engl J Med 291* 6 1974

23 Thomas D B Detection and treatment of severe coagulation disturbances in the neonatal period *Med J Aust 1* 96? 1974

4 Thorburn M J Neonatal death and massive pulmonary haemorrhage in Jamaica *Arch Dis Child 38* 589 1963

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SERUM LYSOZYME ACTIVITY IN CHILDREN WITH HEMATOLOGICAL AND MALIGNANT DISORDERS

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ABSTRACT Moe P J, Haneberg B and Finne P H (Department of Paediatrics University of Tromsø Tromsø and Department of Paediatrics University of Bergen Bergen Norway) Serum lysozyme activity in children with hematological and malignant disorders Acta Paediatr Scand 64 830, 1975 —Pretreatment serum lysozyme activity was high in 2 children with myelomonocytic leukemia 800 and 1 000 μg/ml normal in all 10 cases of acute myelocytic leukemia and subnormal in 21 of 34 cases of acute lymphocytic leukemia Normal values were found in all but one case of acute lymphocytic leukemia during complete remission including 8 cases after all therapy had been discontinued All 8 are still in complete remission Low serum lysozyme activity was found in 5 patients with acute lymphocytic leukemia in complete relapse, this could possibly be helpful in the diagnosis of early relapse Activity was subnormal in 5 of 17 children with malignant tumours and in 3 of 65 cases of various benign hematological disorders

KEY WORDS Lysozyme serum leukemia pretreatment remission relapse malignant tumours

Classification of the acute leukemias on the basis of cell morphology alone may be extremely difficult Even the differential diagnosis between acute leukemia and neuroblastoma with diffuse infiltration of the bone marrow may be a problem especially since normal catecholamine excretion may be found in the disseminated type of neuroblastoma

Thus laboratory studies that may be of assistance in such situation are needed Several studies reported in the literature indicate that measurement of serum lysozyme is useful both in the diagnosis and classification of acute leukemia, (2 3, 4 5) Most of the reported cases have been adults

The serum lysozyme activity was measured in children with various types of acute leukemias and for comparison the activity in sera from children with malignant tumours and with various benign hematological disorders were studied

MATERIAL AND METHODS

Serum lysozyme was measured in 46 untreated children with acute leukemia and in 31 of them in remission as well as in 5 cases in complete relapse All therapy had been discontinued in 8 children still in remission

Studies have also been performed in 17 children with malignant tumours and 65 children with various benign hematological disorders including osteopetrosis and aplastic anemia

The serum lysozyme activity was measured with a modified agar lysoplate technique (1) The results must be compared using the same technique Normal range in 95 children was 67–400 μg/ml with a 95 per cent range of about 50–750 μg/ml

RESULTS AND DISCUSSION

Fig 1 (semilogarithmic scale) clearly demonstrated the difference between cases of acute lymphocytic leukemia (ALL) acute myelocy

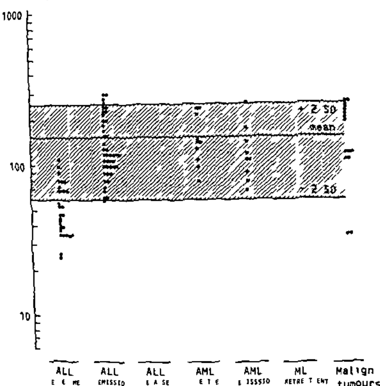


Fig 1 Serum lysozyme activity in children with various hematological and malignant disorders The values including the normal range which is shaded were based on egg white lysozyme as standard

tic leukemia (AML) and monocytic leukemia (ML)

Low pretreatment values were found in 21 of 34 cases of ALL Only 2 of the 34 cases had lysozyme activity above 100 µg/ml One of these 2 had total WBC of 155 000/mm³ Low values were also found in 5 cases of ALL in complete relapse Normal pretreatment values were found in all 10 cases of AML

Very high values were observed in 2 cases originally classified morphologically as paramyeloblast leukemia Both were older children with blast cells containing abundant cytoplasma indicating a poor prognosis They died shortly after therapy had been started The morphological picture was compatible with a diagnosis of myelomonocytic leukemia in both cases and the very high lysozyme activity support the contention that these 2 cases actually belonged to the monocytic types of leukemia (ML) High values have been reported in almost all cases of ML in adults Both ML patients died shortly after admission 9 of 10 cases of AML within 3 years and one of the 2 patients with ALL with lysozyme activity above 100 µg/ml within 4 months Thus in our study there seems to be a relatively good inverse correlation between pretreatment lysozyme activity and response to therapy

Normal values were found in all but one case of ALL during complete remission That child was completely well Later on he had a meningeal episode with slightly increased number of cells in CSF He has now been in complete hematological remission for 4 years Today most cases of ALL remain in hematological remission for years and we have not been able to present sufficient data on cases in partial remission All 5 cases in complete relapse had low lysozyme activity

Studies have so far been performed in 8 cases after all therapy had been discontinued They are all still in complete remission after 4

months to 2 years without therapy The values ranged between 170 and 300 μg/ml in these 8 cases Mean pretreatment level in 6 of the cases was 56 μg/ml and after therapy had been discontinued 230 μg/ml

Subnormal lysozyme activity was found in 6 of 17 children with malignant tumours (Hodgkin s disease and neuroblastoma) and in 3 of 65 cases of various benign hematological disorders Low serum lysozyme activity is, thus, not only seen in ALL None of the 9 patients with low serum lysozyme activity had neutropenia Normal values were however found in 3 children with aplastic anemia in spite of marked neutropenia and in 3 cases of osteopetrosis

REFERENCES

1 Haneberg B & Finne P H Lysozymes in feces from infants and children *Acta Paediatr Scand 63* 588 1974
2 Levi J A Speden J B Vincent P C & Gunz F W Studies on muramidase in hematological disorders *Cancer 4* 939 1073
3 Ohta H & Nagase H Serum urine and leukocyte muramidase (lysozyme) activity in monocytic leukemia and other hematologic malignancies *Acta Haematol Jap 34* 498 1971
4 Perille P E & Finch S C Lysozyme in leukemia *Med Clin N Amer 57* 395 1973
5 Seligman B R Rosner F Panse F & Lee S L *Serum muramidase in acute leukemia Am J Med Sci 264* 69 1972

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RENAL TRANSPLANTATION IN CHILDREN

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ABSTRACT Henriksson C Andersen H J Gustafsson Å and Gelin L E (Department of Surgery I Sahlgren s Hospital Gothenburg Sweden and Department of Pediatrics Odense Hospital Odense Denmark) Renal transplantation in children Acta Paediatr Scand 64 833 1975.—From July 1967 to September 1974 26 kidney transplantations were carried out in 16 children aged 6 to 17 years in Gothenburg The average age at the primary transplantation was 12 years and average body weight 29 7 kg Five patients had familial juvenile nephronophthisis 5 chronic glomerulonephritis 5 chronic pyelonephritis and one bilateral Wilms s tumour Four patients were predialytic Fourteen grafts came from living related donors The surgical technique was standard as was the immunosuppression with azathioprine and cortisone exceptionally antilymphocyte globulin was used Thirteen patients were alive in September 1974 observed 2–65 months 8 with a normal serum creatinine 3 with moderately elevated serum creatinine and 2 on hemodialysis. The 6- and 12 month survivals of patients are 100% and 93% respectively Normal growth and full rehabilitation in recipients of functioning grafts make renal transplantation justified as a therapeutic procedure in terminally uremic children

KEY WORDS Renal transplantation uremia in children Wilms s tumour

Uremia is a relatively rare cause of death in children and the mortality rate among Swedish children is not known exactly In Great Britain mortality in the age group 0–14 years has been estimated at 3–5 per million total population per annum (2) Potter et al (17) estimated in northern California that in their 8 million population 50 children a year would benefit from definite therapy Congenital malformation pyelonephritis with or without malformations and—less common—different forms of acquired irreversible glomerular lesions are considered to be the common causes of uremia

It is the purpose of this communication to report the experience gained at the Department of Transplantation Surgery in Gothenburg Transplantation in children started in 1967 after more than 2 years experience of operation on adults The 16 children described here belong to a total series of 455 patients

MATERIAL

From July 1967 to September 1974 16 children were treated with 26 kidney transplantations Seven grafts were secondary and three were tertiary The children were in the ages 6–17 years at the time of primary transplantation There were 9 girls and 7 boys The body weight at the primary transplantation was 17–41 kg and the average weight 29 7 kg Age sex body weight original disease hemodialysis before transplantation donor source and number of incompatible antigens are shown in Table I Living donors were parents in 14 cases and a sister in one case Two primary grafts and all secondary grafts came from cadavers

The recipients were referred from various pediatric units They were all in a terminal stage of uremia 1 of them om maintenance hemodialysis for 1–14 months Five recipients were nephrectomized bilaterally before the

Table 1 *Clinical details of renal transplant donors and recipients*

Patient no and sex	No of grafts	At the time of transplantation: Age (years) 15 1/12	At the time of transplantation: Body weight (kg)	Original disease	Hemodialysis transpl (months)	Donor	Donor's age (years)	No of incompatible antigens
1 F	I	15 1/12	47	Chronic glomerulonephritis	2	Mother	44	Unknown
	II	15 8/12	41		8	Cadaver	20	2
2 M	I	14 11/12	37	Familial juvenile nephronophthisis	5	Mother	41	1
	II	16 9/12	75		No	Cadaver	24	0
3 M	I	10 2/12	25	Familial juvenile nephronophthisis	No	Father	45	1
	II	10 8/12	23		No	Cadaver	4	0
4 M		10 9/12	39	Chronic glomerulonephritis	2	Mother	49	0
5 F		12 1/12	27	Chronic pyelonephritis	No	Mother	47	1
6 F	I	13 11/12	41	Chronic glomerulonephritis	7	Mother	40	1
	II	15 4/12	40		16	Cadaver	7	3
	III	16 1/12	40		8	Cadaver	16	2
7 M	I	10 11/12	27	Familial juvenile nephronophthisis	7	Mother	36	1
	II	12 9/12	38		0	Cadaver	33	2
	III	12 10/12	34		0	Cadaver	19	2
8 F	I	13 1/12	31	Chronic glomerulonephritis	12	Cadaver	21	0
	II	13 5/12	34		3	Cadaver	51	1
	III	14 4/12	35		13	Cadaver	16	2
9 F		8 10/12	19	Familial juvenile nephronophthisis	No	Father	43	1
10 M		6 5/12	17	Bilateral Wilms tumour	1	Father	33	1
11 F		13 1/12	27	Chronic pyelonephritis	3	Father	45	1
12 M	I	11 3/12	24	Familial juvenile nephronophthisis	No	Mother	45	2
	II	11 8/12	26		2	Cadaver	47	1
13 F		10 4/12	23	Chronic pyelonephritis	14	Cadaver	35	1
14 M		14 1/12	38	Chronic pyelonephritis	3	Mother	49	2
15 F		8 6/12	19	Chronic pyelonephritis	2	Sister	18	0
16 F		11 1/12	35	Chronic glomerulonephritis	1	Mother	31	2

primary transplantation The indication for nephrectomy was glomerulonephritis with hypertension in 4 cases and bilateral Wilms's tumour with subsequent irradiation nephritis in one case

METHODS

In selection of donors compatibility in blood groups and HL A system was considered The histocompatibility testing of the recipients and donors was performed according to a method previously described from this centre (22) The number of incompatible antigens varied from 1 to 3 (Table1)

Operative procedure

All children were operated under general anesthesia with a technique used as standard in adults The kidneys were placed extraperitoneally Because of differences in the calibres of arteries the technique for arterial anastomosis varied In most cases the internal iliac artery was used for end to-end anastomosis to the renal artery The renal vein was in all cases anastomosed end to side to the external iliac vein The ureter was implanted into the bladder in all cases except one where the anastomosis was performed between the recipient's ureter and the pelvis of the transplanted kidney The implantation was done either in the neck of the bladder *ad modum* Girgis & Veenema (8) or in the fundus of the bladder Two of the grafts had double ureters and they were implanted separately Twenty cases had a bladder catheter postoperatively Peritoneal dialysis was given in case of a delay in onset of kidney function after three cadaveric transplantations After the operation the children were brought back to the transplant ward for recovery

Immunosuppression

The standard immunosuppressive treatment was azathioprine (Imurel®) in a dose of 2–3 mg/kg body weight/day and prednisolone 100 mg initially and thereafter tapering

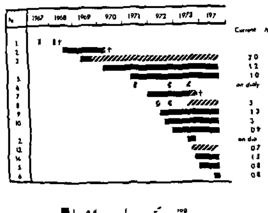


Fig 1 Children treated with renal transplantation

the dose to about 1 mg/kg/day after 14 days Usually azathioprine (Imurel®) was given a few days preoperatively Patient no 1 was treated with extracorporeal irradiation of the blood according to a method described previously from this centre (16) Rejection episodes were treated by actinomycin C (Sanamycin®) 1 000 gamma and local irradiation in a dose of 450 rad The dose of prednisolone was increased temporarily during the rejection episodes *Rheomacrodex® infusions were given routinely* during the first 3 postoperative days this treatment being repeated about twice a week during the rest of the stay in hospital To prevent fungus infections postoperatively the patients received Mycostatin® solution methyl rosaniline solution and sodium bicarbonate tablets

Rejection

Signs of rejection were fever enlargement of the graft and lowering of urine secretion In comparison with adults an increase in the serum creatinine in the children was a *relatively late symptom of rejection In order to investigate* an increase in serum creatinine or to determine the cause of hypertension an angiography of the graft was performed (15) Altogether 28 angiographies were made in this series *No local or general complication from this* procedure was seen In three cases needle biopsy was made in order to establish a rejection diagnosis

RESULTS

Patient and graft survival time and current graft functioning are shown in Fig 1

Patient and graft survival

Thirteen of the 16 patients were alive in September 1974 with an observation time of 2-65 months 8 observed for more than 12 months Two patients were kept on maintenance hemodialysis after irreversible rejection of a second and a third graft Three patients died from sepsis after retransplantation The patient survival rate 6 months after first transplantation was 100% (15/15) After 1 year 93% (13/14) were alive The primary graft survival rate after 6 months was 73% (11/15) One year graft survival was 64% (9/14) Three kidney grafts never functioned adequately (two second grafts and one third graft) The serum creatinine at the end of the observation time is evident from Fig 1 Three patients had a serum creatinine elevation because of slow chronic rejection

Elevated blood pressure was a rule in the post transplantation course which was partly explained by the prednisolone therapy it declined when cortisone doses were diminished Four children had persistent hypertension despite good renal function and having their kidneys removed These patients required treatment with antihypertensive drugs In none of the cases was renal artery stenosis found to be the cause of hypertension *Significant bacteriuria* appeared postoperatively in all cases Patient 5 had urinary infections with pseudomonas which were highly resistant to therapy The cause of uremia in this case was chronic pyelonephritis after many attempts at reconstructive surgery After removal of the patient s own kidneys no less than four ureter stumps were left The recurrent infections disappeared after removal of all four ureter stumps and the urine remained sterile for a year Eight children had on one or more occasions biochemical evidence of *liver engagement* Elevation of glutamic oxaloacetic transaminase and/or alkaline phosphatase was noted Four of these children had a positive Australia antigen test at the same time Two children also developed clinical evidence of hepatitis

Skeletal complications were seen in 3 recipients One boy developed pronounced osteoporosis postoperatively without obvious fractures or deformations This patient had to have a high dose of cortisone for a long time Two patients had skeletal changes preoperatively which were considered to be renal ra

chitis These 2 patients improved greatly when renal function was restored Aseptic bone necrosis was not observed in any of the cases

Gastrointestinal complications were a major problem in patient no 2 who developed a bleeding peptic ulcer after retransplantation He died in a severely uremic state *Diabetes mellitus* or *cataract* has so far not been observed A normal linear *growth rate* among the children of this series reoccurred after a successful transplantation (4) It was found that all patients were in the low normal range of growth due to preoperative renal insufficiency The first 2 patients in this series did not increase in growth because they had a satisfactory graft function only for short periods *Menarche* has occurred in the 4 girls who were transplanted before puberty (cases 5 6, 8 and 11)

All the patients were of school age at the time of operation Eleven had been on hemodialysis for varying lengths of time before the transplantation The dialysis treatment interfered with their school attendance Five patients progressed well while the other 6 had difficulties in keeping up with lessons Two patients have returned to hemodialysis and another 3 patients were hampered due to chronic rejection while 7 were fully rehabilitated They have marks in school which are as good as or better than the average of their classmates One boy has left school and is working full time as a mechanic and combines this with university studies

DISCUSSION

Many pediatricians nephrologists and transplant surgeons have so far been reluctant to accept children as candidates for treatment with dialysis and transplantations The reasons for this have partly been technical partly ethical (18) Others however started quite early to actively treat children who had terminal uremia (1 7 12 13, 21) Subsequently it became possible to solve most of the technical problems with dialysis even for infants (3, 20) and it is thus now possible to maintain childre with total renal insufficiency in a satisfactor condition Although hemodialysis in hospital or at home is acceptable to most patients i means a heavy burden on the patients an their families Most authors agree that a rena transplantation should be the ultimate goal i the treatment of terminal renal failure i children For instance this has recently bee emphasized in a recommendation approve by the Council of the American Society o Pediatric Nephrology (10) From a surgica point of view the procedure of renal transplan tation in children is the same as that in adult (14) The survival rate of children who hav received a transplant has been found to be as good as or even better than the corresponding rate of adults Thus the latest report of the European Dialysis and Transplant Association (19) which includes 179 pediatric renal transplantations performed before December 31 1972 showed a survival rate of 86 83 72 and 66% 1 2 3 and 4 years after the transplantation In 1971 Lilly et al (13) reported on a series of 57 children with 70% survival after 1½–2¾ years Fine & Grushkin (6) in a series of 81 children transplanted during the 6 years up to February 1973 report 65 surviving with functioning grafts 9 were dead and 7 were on hemodialysis In the present series of 16 patients similar results were obtained Two of the children who died were the first ones treated and both died after a secondary transplantation—one shortly after his third transplantation because of severe pneumonia and sepsis The importance of the tissue typing on graft and patient survival could not be assessed in this material as the sub groups were too small

The degree of rehabilitation is a matter of as much concern as the rate of survival In this series eight patients out of the 13 survivors seem to be well rehabilitated and able to fulfil normal activity with regard to their age This is in good correspondence with the results of Korsch et al (11) who found that 28 out of 35 children transplanted one to five years earlier

were attending appropriate schools Although some increase in height has been achieved in several of the patients in this series the degree of growth in children after renal transplantation remains a major problem This problem has recently been analysed by Grushkin et al (9) who concluded that the most important factor affecting growth is the bone age at the time of transplantation These authors found that 10 out of 14 patients with a bone age of 12 years or less achieved significant growth and 6 of them grew at a normal rate On the other hand only one patient out of 12 with a bone age more than 12 years grew significantly The influence of steroid treatment was less obvious but if the dose of prednisone for long term maintenance therapy exceeded 10 mg/m²/day growth was likely to be suppressed

In the present material some of the patients have had hypertension without such reasons as renal artery stenosis high steroid doses or chronic rejection All patients have had cortisone doses which were lower than those which have been given at some other centres (7 13 17) about 0 2 mg/kg/day as a maintenance dose of Prednisolone® or Medrone daratum® (methylprednisolone) The blood pressure was lower and easier to control after bilateral nephrectomy A high frequency of unexplained hypertension among children with renal transplants has been mentioned by some authors (5 14) It might be suspected that this type of hypertension could have its origin in an increased renin secretion caused by the great demand of blood flow for perfusion of an adult kidney in a small child

REFERENCES

1 Belzer F O Schweitzer R T Holliday M Potter D & Kountz S L Renal homotransplantation in children *Am J Su g 1?4* ??0 197
British Paediatric Association unpublished memorandum quoted in Barrat T M Renal failure in the first year of life *B Med Bull* ?7 115 1971
3 Buselmeier T J Santiago E A Simmons R L Najarian J S & Kjellstrand C M Arteriovenous shunts for pediatric hemodialysis *Surgery 70* 638 1971
4 Engstrom I Karlberg P Klackenberg G Klackenberg Larsson I Lichtenstein H Svennberg I & Taranger J Tillvaxtdiagram for langdvikt och huvudomfång från fodelsen till 18 år *Lakartidningen 70* ?960 1973
5 Fine R N Edelbrock H H Brennan L P Grushkin C M Korsch B M Riddell H Stiles Q & Lieberman E Cadaveric renal transplantation in children *Lancet I* 1087 1971
6 Fine R N & Grushkin C M Hemodialysis and renal transplantation in children *Clin Nephrol I* ?43 1973
7 Fine R N Korsch B M Stiles Q Riddell H Edelbrock H H Brennan P Grushkin C M & Lieberman E Renal homotransplantation in children *J P diatr 76* 347 1970
8 Girgis A S & Veenema R J Triangular flap ureterovesicoplasty a new technique for the correction of ureteral reflux a preliminary report *J U ol* 94 ?33 1965
9 Grushkin C M & Fine R N Growth in children following transplantation *Am J Dis Child 125* 514 1973
10 Grushkin C M Lewey J E Cole B Holland N Lewey P & Potter D Hemodialysis and renal transplantation in children the role of the pediatric nephrology team *Pediatrics 54* 864 1974
11 Korsch B M Negrete V F Gardner J E Weinstock C L Mercer A S Grushkin C M & Fine R N Kidney transplantation in hildren psychosocial follow-up study on child and family *J Pediatr* 83 399 1973
12 LaPlante M P Kaufman J J Goldman R Gonick H C Martin D C & Coodwin W E Kidney transplantation in children *Pediatrics* 46 665 1970
13 Lilly J R Giles G Hurwitz R Schroter G Kakagi H Gray S Penn I Halgrimson C G & Starzl T Renal homotransplantation in pediatric patients *Pediatrics 47* 548 1971
14 Najarian J S Simmons R L Tallent M B Kjellstrand C M Buselmeier T J Vernier R L & Michael A F Renal transplantation in infants and children *Ann Si rg 174* 583 1971
15 Nilsson A E Jacobsson B Bergentz S E & Westberg G Angiography of the transplanted kidney *Scand J Urol Nephrol 2* 46 1968
16 Persson B Rosengren B Olander R Bergentz S E Gelin L E & Hood B Preoperative extracorporeal irradiation of the blood as adjunctive immunosuppression in human renal transplantation *Ann Surg 174* 80 1971
17 Potter D Belzer F O Rames L Holliday M A Kountz S L & Najarian J S The treatment of chronic uremia in childhood I Transplantation *Pediatrics 45* 43? 1970
18 Riley C M Thoughts about kidney homotransplantation in children *J Ped at 65* 797 1964
19 Scharer K Brunner F P von Dehn H Curland H J Harlen H & Parsons F M Combined report on regular dialysis and transplantation of children in

Europe 1972 *Proc Eur Dial Transplant Assoc 10* 58 1973
20 Shideman J R Meyer R M Buselmeier T J Mauer S M & Kjellstrand C M Hemodialysis of very small pediatric patients Technical problems *Dial Transplant 2* 25 1973
21 Starzl T E Marchioro T L Porter K A Faries T D & Carey T A The role of organ transplantation in pediatrics *Pediatr Clin North Am 13* 381 1966
22 Storm B Graft and patient survival after primary cadaver kidney transplantation with special regard to compatibility in the HL A system *Acta Chir Scand* Suppl *437* 1973

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SYSTOLIC TIME INTERVALS IN NEWBORN INFANTS

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ABSTRACT Hedvall G (Department of Paediatrics I Östra sjukhuset University of Goteborg Goteborg Sweden) Systolic time intervals in newborn infants Acta Paediatr Scand 64 839 1975 —Systolic time intervals calculated from the carotid artery pulse curve have been used for some time to assess left ventricular performance Normal values have been established for adults and older children but few investigations have been made on newborn infants generally with partly contradictory results Therefore a study has been undertaken in 29 normal newborn infants 1–119 hours old and 9 infants 2–3 months old to establish normal values for the different intervals They were found to differ from those reported for adults and older children most clearly seen in the relatively long preejection period (PEP) Statistically significant differences were found between the PEP of 20 infants below 47 hours of age and 9 infants 2–3 months old PEP 82 ms and 68 ms respectively ($p<0.01$) the same is true of the quotient PEP/LVET (left ventricular ejection time) which was found to be 0 41 and 0 35 resp ($p<0.01$) By adult standards this would mean impairment of left ventricular function A possible explanation of this could be a difficulty for the left ventricle to cope with the systemic circulation during the first days of life even in normal newborns a difficulty not fully compensated for even at 3 months of age

KEY WORDS Newborns left ventricular function carotid artery pulse curve

During the last decade there has been an increasing interest in the use of systolic time intervals in the analysis of left ventricular performance The various intervals have usually been measured from a carotid artery pulse curve recorded simultaneously with an ECG and PCG lead The intervals most commonly used are those shown in Fig 1 LVET (left ventricular ejection time) is measured from the beginning of the rapid upstroke of the carotid pulse curve to the incisura of the descending portion of the curve Q S II (the electromechanical systole) is measured from the beginning of the Q wave of the ECG to the first vibrations i e the aortic component of the second heart sound in the PCG The pulse delay i e the difference of 5–10 ms between the second heart sound and the incisura of the descending pulse curve is not clearly shown in this figure

PEP (the preejection period) is calculated by subtracting LVET from Q S II It includes both the conduction dealy in the left ventricle and the isovolumetric contraction time (ICT) ICT can be measured as the interval between the first vibrations of the first heart sound and the upstroke of the carotid pulse curve with correction made for the pulse delay

Normal values for these different systolic intervals have been established by Weissler (9) and others and the various intervals have been found to correlate with heart rate The isovolumetric contraction time (ICT) would seem to be the best indicator of overall left ventricular performance i e left ventricular contractility but in clinical application and in the absence of left ventricular bundle branch block PEP (the pre ejection period) gives a useful measure of left ventricular perform

Table I *Some systolic time intervals in children from the literature*

PEP Preejection period ICT Isovolumetric contraction time LVET Left ventricular ejection time

	Age	n	PEP (ms)	ICT (ms)	PEP/LVET
Golde et al	1 6 y ♀ (mean)	30	34	14	0 19
	13 75 y ♀ (mean)	30	93	33	0 33
Harris et al	1 d –4 d	50		32	
	1 y – 14 y	49		26	
Hernandez et al	2 d – 1 y	77			0 35–0 76
Park et al	prem 5 h – 4 w	25			
	term 2 h – 5 d	85	52–75		
	1 m – 11 m	40			
Spitaels et al	1 m – 15 y	76			0 31

ance The quotient PEP/LVET has proved of value in patients with impaired left ventricular function most clearly seen in primary myocardial disease (8) where PEP is prolonged while LVET is shortened In these patients thus PEP/LVET is prolonged from the normal 0 35 in adults and this ratio correlates well with cardial output (l/min/m) and stroke volume (ml/m) As it does not vary with heart rate this quotient is especially useful when comparing data from different patients

Normal values for children have been established by Golde et al (1) who examined 390 children in different age groups 1 5 to 13 5 years old These authors found the quotient PEP/LVET in the youngest age group to be only 0 19 as compared with 0 33 in the 13 5 years old (calculated from the values given in the reference quoted where LVET/PEP the systolic quotient is presented) The ICT in the youngest group is 14 ms and in the 13 5 years old 32 ms

Harris et al 1964 (2) studied the systolic intervals in 50 normal full term infants 1–4 days old 47 premature infants 1–33 days old and 49 older children 1–14 years old ICT was found to be shorter in children than in adults and not related to heart rate But the group of term infants (none older than 4 days) had slightly higher ICT values—32 ms—than the two other groups both of whom had ICT 26 ms

Hernandez et al 1972 (3) have found in 77 normal children 2 days to one year old a range of PEP/LVET 0 35 to 0 76

Steinfeld et al 1969 (7) and Park et al 1973 (5) have determined the normal systolic time intervals in 85 full term infants 8 hours to 5 days old and in 25 premature infants 5 hours to 4 weeks old plus 40 infants 1 to 11 months old They found no statistically significant difference between these three groups The PEP values were between 52 and 75 ms and did not correlate with heart rate although the LVET values did They also studied *infants in cardiac* failure and found in those with myocarditis or endocardial fibroelastosis prolonged PEP values

Recently Spitaels et al (6) have found a PEP/LVET of 0 313 in children without cor

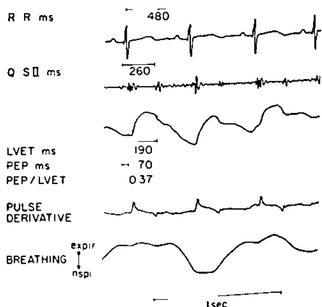


Fig 1 Simultaneous tracings of ECG lead II PCG and the carotid pulse curve showing how the different systolic intervals are measured and calculated The pulse derivative and respiration are also registered The abbreviations are explained in the text

Table 2 *Mean systolic time intervals in five different age groups*

For abbreviations see Table 1

Age	*n*	R-R (ms)	Q-S II (ms)	LVET (ms)	PEP (ms)	PEP/LVET	
0–3 h	11	524	83	203	80	0 39	mean
		74	13	10	8	0 04	S D
24–47 h	9	519	80	196	84	0 43	mean
		70	0	17	10	0 06	S D
48–71 h	11	559	277	200	77	0 39	mean
		43	16	13	8	0 04	S D
96–119 h	10	516	272	197	73	0 37	mean
		36	11	10	6	0 03	S D
2 3 m	9	415	259	192	68	0 35	mean
		43	15	15	2	0 03	S D

relation to HR or age Their children were 1 month to 15 years old

The results from the literature quoted above and summarized in Table 1 are partly contradictory The author therefore wanted to analyze the systolic time intervals in normal newborn infants separated into different age groups—first second third and fifth day of life and followed sequentially as far as possible Although the main purpose of this study was to analyse the newborn circulation from this particular point of view it was considered of interest to include a small group of older infants 2–3 months old

MATERIALS AND METHODS

Twenty nine normal newborn infants were studied in different age groups during the first second third and fifth day of life Some of them have been followed sequentially making the total number of records in newborns 41 In addition another group of 9 infants was studied at 2 to 3 month of age The 9 newborn infants were all *full term* with uneventful pregnancy and birth (two of them delivered by caesarean section for maternal reasons) All infants were kept in the normal nursery and were given routine care No murmurs were heard *at the time of the* study Care was taken to study the infants shortly after feeding relaxed and sleeping well covered up in their usual cot

The carotid pulse curve was recorded with a specially designed plastic funnel (10) connected with an air containing plastic tubing to a transducer Elema Schonander EMT 510C which has an almost flat frequency response of 0 05–30 Hertz and a time constant of 1 9–3 8 sec ECG lead II was recorded simultaneously with one or more PCG leads The respiration was recorded in the manner previously described from this department using the impedance plethysmographic method (4) An ink jet oscillograph was used (Elema Schonander Mingograf 81)

A paper speed of 100 mm/s was used and the intervals read to the closest 5 ms For each child 10 heart beats were analysed for the various systolic time intervals as shown in Fig 1 R-R interval LVET and Q-S II were measured and PEP calculated by subtracting LVET from Q-S II The quotient PEP/LVET was then calculated for each heart beat and the mean value for each patient was calculated for each of these five different parameters The older children (2–3 months old) were taken randomly from a well baby clinic and the registrations made in a similar way excluding respiration monitoring These infants were not sedated they were kept on an examination table lightly covered up and with their mothers sitting beside them From this group of infants 10 heart beats were analysed for each one in the same manner as shown above This group of older children is small owing to the practical difficulties in getting acceptable recordings in healthy infants of this age without sedation Nevertheless the results from this group can be used and compared with those of the other groups if due care is taken in the statistical analysis

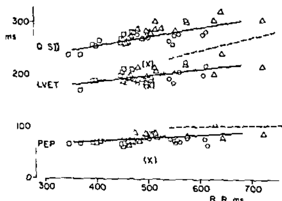


Fig 2 Correlation between the systolic time intervals and heart rate expressed as R-R interval (only first values from each baby) Δ=0–47 h old O=48–119 h old □= 2–3 m old Regression lines ——

Regression equations

Q-S II $y = 186 + 0.17$ R-R $r = 0.78$

LVET $y = 137 + 0.12$ R-R $r = 0.66$

PEP $y = 48 + 0.06$ R-R $= 0.49$

Regression lines for LVET and PEP in adults according to Weissler (8)

(x)=mean values of Q S II LVET and PEP for 1½ year old infants according to Golde (1)

Table 1. *Some systolic time intervals in children from the literature*

PEP Preejection period, ICT Isovolumetric contraction time, LVET Left ventricular ejection time

	Age	*n*	PEP (ms)	ICT (ms)	PEP/LVET
Golde et al	1.6 y ♀ (*mean*)	*30*	*34*	*14*	*0.19*
	13.75 y ♀ (mean)	30	93	33	0.33
Harris et al	1 d – 4 d	50		32	
	1 y – 14 y	49		26	
Hernandez et al	2 d – 1 y	77			0.35–0.76
Park et al	prem 5 h – 4 w	25			
	term 2 h – 5 d	85	52–75		
	1 m – 11 m	40			
Spitaels et al	1 m – 15 y	76			0.31

ance. The quotient PEP/LVET has proved of value in patients with impaired left ventricular function, most clearly seen in primary myocardial disease (8) where PEP is prolonged while LVET is shortened. In these patients thus PEP/LVET is prolonged from the normal 0.35 in adults, and this ratio correlates well with cardial output (l/min/m²) and stroke volume (ml/m²). As it does not vary with heart rate, this quotient is especially useful when comparing data from different patients.

Normal values for children have been established by Golde et al. (1) who examined 390 children in different age groups 1.5 to 13.5 years old. These authors found the quotient PEP/LVET in the youngest age group to be only 0.19 as compared with 0.33 in the 13.5 years old (calculated from the values given in the reference quoted where LVET/PEP, the systolic quotient, is presented). The ICT in the youngest group is 14 ms and in the 13.5 years old 32 ms.

Harris et al. 1964 (2) studied the systolic intervals in 50 normal full term infants 1–4 days old, 47 premature infants 1–33 days old and 49 older children 1–14 years old. ICT was found to be shorter in children than in adults and not related to heart rate. But the group of term infants (none older than 4 days) had slightly higher ICT values—32 ms—than the two other groups, both of whom had ICT 26 ms.

Hernandez et al. 1972 (3) have found in 77 *normal children 2 days to one year old* a range of PEP/LVET 0.35 to 0.76.

Steinfeld et al. 1969 (7) and Park et al. 1973 (5) *have determined the normal systolic time intervals in 85 full term infants 8 hours to 5* days old and in 25 premature infants 5 hours to 4 weeks old, plus 40 infants 1 to 11 months old. They found no statistically significant difference between these three groups. The *PEP* values were between 52 and 75 ms, and did not correlate with heart rate, although the LVET values did. They also studied infants in cardiac failure and found in those with myocarditis or *endocardial fibroelastosis prolonged PEP* values.

Recently Spitaels et al. (6) have found a PEP/LVET of 0.313 in children without cor

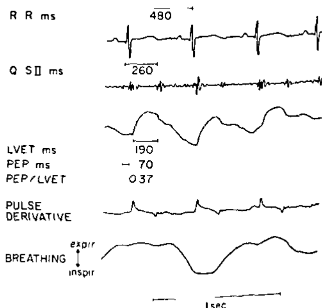


Fig. 1. Simultaneous tracings of ECG lead II, PCG and the carotid pulse curve, showing how the different systolic intervals are measured and calculated. The pulse derivative and respiration are also registered. The abbreviations are explained in the text.

Table 3 *Systolic time intervals in two groups of babies at less than 47 hours and at 2–3 months of age*
For abbreviations see Table 1

Age	n	R–R (ms)	Q–S II (ms)	LVET (ms)	PEP (ms)	PEP/LVET	
0–47 h	0	622	782	700	82	0 41	mean
		77	16	14	9	0 05	S D
2–3 m	9	415	259	192	68	0 35	mean
		43	15	15	2	0 03	S D
Difference		$p<0.01$	n s	n s	$p<0.01$	$p<0.01$	

cant differences between the first few days in this respect as seems to be indicated by the present data this might be attributable to the closure of the ductus arteriosus The discrepancies between these results and those of some other authors are obvious and some explanations of them can be given partly in different techniques partly in the very low number of newborns that are included in some of them

The material of Golde et al (1) does not include newborns the mean age in their youngest age groups is 1 6 years These authors calculate PEP as the sum of Q–S I and ICT which gives the same result as Q–S II minus LVET

Harris et al (2) found higher ICT values in newborns than in older children They measured LVET from the axillary pulse curve the mechanical systole from the PCG the interval between the first and the second heart sound ICT was calculated by subtracting LVET from the S I–S II interval S I is not considered to be a good reference point as it is often difficult to determine exactly from the PCG the first vibrations of the first sound (9)

Hernandez et al (3) have found high values for PEP/LVET in their infants 2 days to 1 year old but one does not know how these values were distributed during the first year of life A Doppler ultrasound technique was used for the registrations of the carotid pulse curve but we do not know exactly how the calculations were made

Park et al (5 7) did not find any differences between the newborn infants and the older ones up to 11 months of age They used instead of the carotid pulse curve an apex cardiogram registered at the left sternal border It is possible to calculate the PEP from an apex cardiogram but this is fraught with technical inaccuracies (9) The authors have tried to verify their measurements by simultaneous recordings of central aortic pulse curves and apex cardiogram but this was done in 17 infants of whom 12 had congenital heart disease Moreover an apex registration from a newborn infant taken at the left sternal border seems to represent right rather than left ventricular performance! An ECG taken at the same place as the cardiogram can show which ventricle is registered (6)

Spitaels et al (6) have calculated PEP and LVET in the same manner as the present author Their value of PEP/LVET of 0 31 at all ages is more in accordance with the present results than are those of Golde et al No newborn infants are included however in their study the youngest one being 1 month old and only 9 below 13 months of age

The present results demonstrate that the systolic time intervals and particularly the quotient PEP/LVET in newborn infants differ from those reported in older infants and children This suggests a difficulty for the left ventricle to cope with the systemic circulation during the first days of life even in normal infants This finding can help our understand

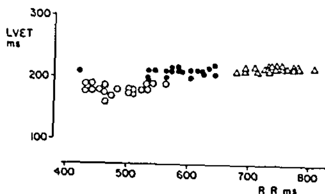


Fig 3 Three individual babies do not change their LVET despite considerable changes of heart rate (mostly due to respiration) Δ=8 h old ●=52 h old O=106 h old

RESULTS

Table 2 shows the mean values and standard deviations of the different systolic time intervals in the five age groups The total number of observations is 50 from 38 different infants Fig 2 shows the correlation between heart rate (expressed as R–R interval) and Q–S II LVET and PEP respectively in the 38 infants (only first values from each baby) There is no correlation between the quotient PEP/LVET and R–R interval (not shown) In this as in the following figures, two separate groups of infants are specially indicated the 9 infants 2–3 months old and the 20 infants below 47 hours of age (the youngest ½ hour old) The regression lines for LVET/R–R and PEP/R–R in adults according to Weissler (9) can also be seen in this figure Quite striking is how individual infants can vary their heart rate over a wide range without changing LVET at all Fig 3 shows three individual infants of different ages where this tendency is very clear the changes in the heart rate being respiration dependent

Fig 4 shows the quotient PEP/LVET in the different age groups with their mean values (see also Table 2) It is shown also how some infants are followed twice during the first few days There seems to be a tendency for the PEP/LVET to be highest on the second day of life and then to fall slowly, but this material does not permit any statistical analysis in this respect However if the 9 infants 2–3 months old are compared with the 20 infants below 47 hours of age using only first values from each infant highly significant differences are obtained The mean values for the systolic time intervals from these two groups are shown in Table 3 The difference of the PEP and the quotient PEP/LVET of these two groups is highly significant

DISCUSSION

The present material provides values for the various systolic time intervals at variance with some earlier results (5 7) and strikingly different from those found in older children and adults (1 8)

The most important finding seems to be that the PEP and the quotient PEP/LVET which is not rate dependent are higher in the newborn infants than in infants 2–3 months old In adults this quotient is high in heart failure most typically with myocardial disease indicating a low contractility of the left ventricle Thus compared with adult standards the left ventricle function in newborn infants seems to be relatively impaired It is tempting to correlate this with the fact that at birth the left ventricle suddenly is confronted with the whole systemic circulation Before birth both ventricles have worked in parallel and the systemic resistance has been low thanks to the placental circuit If there are any signifi

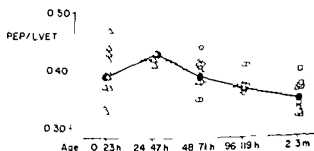


Fig 4 The quotient PEP/LVET is shown in the various age groups individual babies as well as the group means (●—●) connects sequential values from different observations on the same child

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MENARCHEAL AGE IN NORTHUMBERLAND

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ABSTRACT Roberts D F Danskin M J and Chinn S (Department of Human Genetics University of Newcastle upon Tyne and MRC Clinical Research Centre Harrow England) Menarcheal age in Northumberland Acta Paediatr Scand 64 845 1975.—A second survey of age at menarche in north-east England how it is affected by family environment and how it affects physique was carried out in 1969–70 on a large sample of schoolgirls in the suburbs of Newcastle Age at menarche shows no independent effect of social class or of position in sibship but is strongly influenced by the size of family in which a girl grows up Menarche is associated with pronounced increments in height and weight in the presence of which no consistent effects of variables of the family environment on physique can be clearly identified The results are very similar to those from the first study in South Shields County Durham

KEY WORDS Menarcheal age social class family size physique

In 1967 was carried out a first survey of menarcheal age in north east England (8) In schoolgirls aged 9 to 16 in the industrial urban community of South Shields County Durham menarcheal age was estimated by logit analysis The results provided something of a surprise for the overall median age (13 43+0 05 years) was later by one third of a year than that in the last reported sample from Britain analysed by a similar method (9) Two possible reasons were suggested for this delay First it was thought that it might perhaps indicate an interruption or termination of the secular trend to earlier menarche which had characterised British samples like those elsewhere in Europe over a long period Alternatively it was suggested that it might be a reflection of the characteristics of the sample drawn from an urban industrial area where there was still much economic hardship and resulted from some environmental retardation of maturation The South Shields results were subsequently shown (1) to be concordant with the findings of a series of cross sectional studies by Swansea University students which indicated that the secular trend to earlier age at menarche had ceased and had possibly reversed It was therefore important to examine another sample from north east England The present study was made in schoolchildren in south east Northumberland from middle class suburban areas of Newcastle upon Tyne in which it was thought that there would be less economic hardship than in South Shields The survey was undertaken in the period July 1969 to February 1970

MATERIAL AND METHODS

In the Whitley Bay area eight schools were visited and in Gosforth five Both Whitley Bay and Gosforth are suburbs of Newcastle primarily better class dormitory areas for those who work in the City and elsewhere on Tyneside In all 13 J girls were investigated but the 14 twins among them were then excluded leaving 1 307 For each girl was recorded the date of examination her date of birth her height weight whether she had attained menarche and if so the date (recorded as the year and month of onset)

ng of the important transitional period of the circulation in newborn infants

ACKNOWLEDGEMENT

With the technical assistance of Marta Boda

REFERENCES

1 Golde D & Burstin L Systolic phases of the cardiac cycle in children *Circulation 42* 1029 1970
2 Harris L C Weissler A M Manske A O Danford B H White G D & Hamill W A Duration of the phases of mechanical systole in infants and children *Am J Cardiol 14* 448 1964
3 Hernandez A Klint R B Okamoto G A & Goldring D Non invasive technique for evaluation of cardiac function in infants and children *Pediatr Res 6* 345/85 (abstr) 1972
4 Olsson T & Victorin L Transthoracic impedance With special reference to newborn infants and the ratio air to fluid in the lungs *Acta Paediatr Scand* Suppl *207* 37 1970
5 Park S C Steinfeld L & Dimich I Systolic time intervals in infants with congestive heart failure *Circulation 47* 1281 1973
6 Spitaels S Arbogast R Fouron J C & Davignon A The influence of heart rate and age on the systolic and diastolic time intervals in children *Circulation 49* 1107 1974
7 Steinfeld L Park S & Dimich I Right ventricular apex cardiography in infants (abstr) *Circulation 40* (suppl III) III 195 1969
8 Weissler A M Harris W S & Schoenfeld C D Systolic time intervals in heart failure in man *Circulation 37* 149 1968
9 Weissler A M Lewis R P & Leighton R F The systolic time intervals as a measure of left ventricular performance in man *In* P N Yu & J F Goodwin (eds) *Progress in Cardiology* Lea & Febiger Philadelphia 1972 p 155
10 Wikstrand J Wallentin J & Nilsson K (to be published)

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Table 3 *Distribution of girls by family size and birth year*

Birth Year	Family size 1	2	3	4	5	6	7	8	9	10	Mean±S E	Total
1952	1	0	1	0	0	0	0	0	0	0	2 0 –	2
1953	2	7	14	6	2	0	1	0	0	0	3 09 212	32
1954	14	55	41	25	13	5	3	0	1	0	3 01 114	157
1955	25	94	85	50	21	13	8	1	1	0	3 10 085	298
1956	18	101	89	41	13	6	4	3	1	0	2 95 081	276
1957	14	91	66	30	6	4	6	3	0	1	2 91 097	221
1958	10	60	52	18	7	7	3	1	3	0	3 05 123	161
1959	10	44	39	13	7	2	0	0	1	1	2 85 129	117
1960	2	12	14	11	1	3	0	0	0	0	3 14 184	43

bution Having fitted the complete model the main effects of the qualitative variables and in one analysis an interaction between two of them were investigated by taking groups of variables in turn deleting from the appropriate overall regression the terms to be examined and assessing the significance of the additional residual heterogeneity that resulted

RESULTS

Exploratory Analysis

The first exploratory analysis identified the most profitable variables for detailed investigation Here were screened effects of school family size family position combinations of family size and position parents social class and the mid point of the girl s age group Despite the number of girls in the survey there were clearly too many possible sub-categories for useful investigation so to give an analysis of manageable size the variables were restricted Only social classes 1–5 were considered and subjects whose fathers were unemployed deceased or parents separated were excluded Girls of social classes 1 and 2 were combined Family size was classified as 1 (no siblings) 2 3 4 5 or more Family position was classified into first last or middle The three junior schools in which only one girl had attained menarche were omitted All possible combinations of family size and position were also included as well as the main effects of each variable

The results of this analysis are given in Table 4 This shows first that the interactions between family size and position can be ignored for if this is done the increase in deviance (χ^2 5 6 d f 5) from the general model is insignificant Using then for comparison the model incorporating main effects only removal jointly of family size and family position produced an increase in deviance which was not quite significant at the 5% level Their combined effect is due primarily to family size rather than family position The difference between the increase in deviance on deleting both variables and the sum of the increases produced by deleting the variables separately is due to the non orthogonality of the variables By the same procedure the effect of social class considered alone approached

Table 4 *First analysis—values of deviance*

	Deviance (χ^2)	d f
General model (Main effects of school social class family size family position and interaction of family size and position)	571 0	635
Increase due to deleting interaction from model	5 6	5
Main effects model	576 6	640
Increase due to constraining hypotheses		
No family size effect	6 7	4
No family position effect	0 7	2
No family size or position effect	12 2	6
No social class effect	7 6	3
No school effect	5 3	6
No school or social class effect	1 0	9

Table 1 *Distribution of girls by family size and position in family*

Position in family	Family size 1	2	3	4	5	6	7	8	9	10	Total
1	96	237	144	55	17	3	3	–	–	–	555
2	–	227	147	54	15	10	2	1	–	–	456
3	–	–	110	52	15	10	6	–	1	–	194
4	–	–	–	33	11	4	6	3	3	–	60
5	–	–	–	–	12	10	2	1	1	–	26
6	–	–	–	–	–	3	2	2	1	–	8
7	–	–	–	–	–	–	4	1	–	–	5
8	–	–	–	–	–	–	–	–	–	–	0
9	–	–	–	–	–	–	–	–	1	1	2
10	–	–	–	–	–	–	–	–	–	1	1
Total	96	464	401	194	70	40	25	8	7	2	1 307

Also recorded were social data of family size (as indicated by the number of sibs) the girl s position in the family and the social class defined by father s occupation as in the Registrar-General s classification It was noted where relevant whether the family was in economic hardship and received National Assistance whether the father was unemployed or in prison or deceased and whether the parents were divorced or separated Half sibs step-sibs and foster sibs were all included in the measure of family size

Details of the sample are set out in Tables 1–3 Though the majority of girls come from small families there are appreciable numbers from sibships of four or more (Table 1) Apart from families in domestic hardship there is a conspicuous trend to decreased family size with higher social class the greatest mean family size occurs where the father is unemployed (Table 2) It is interesting that in this area the fathers of 2 7% of girls were unemployed of 2 2% were dead while the parents of a further 2 5% of girls were separated or divorced The corresponding figures in the South Shields sample were 3 0% 3 2% and 1 4% The two samples do not appear to differ greatly in these respects The sample spanning only 9 years of birth shows no evidence of any secular change in family size (Table 3)

The method of analysis is almost identical to that used on the South Shields data the criterion for assessing goodness of fit being slightly different The girls were divided into 6-month age groups and by the variables to be investigated in each section of the analysis A logit transformation $Y = \log (p/1-p)$ was applied to the proportion which had attained menarche in each age group This transformed proportion was assumed to depend on a linear function of age and of the main effects of all the other variables investigated Each variable other than age was replaced by several mutually exclusive 0 1 variables one for each alternative value so that it was not necessary to assume for instance the effect of family size on age at menarche to be linear Maximum likelihood estimates of the regression parameters were obtained by iteration and the overall goodness of fit was assessed by the log likelihood ratio (l) of this regression model to the complete model in which since there are as many parameters as data points the fit is exact The deviance ($-2L$) is asymptotically equivalent to the usual χ^2 test for goodness of fit and has also an approximate χ^2 distri

Table 2 *Distribution of girls by family size and social class*

	Family size 1	2	3	4	5+	Mean size ±S E	Total no of girls
Social class							
1	2	19	19	5	2	2 72 0 14	47
2	18	77	77	40	11	2 81 0 07	223
3	57	283	218	92	64	2 82 0 05	714
4	9	43	41	32	33	3 49 0 14	158
5	2	18	21	11	16	3 57 0 20	68
Father unemployed	0	6	9	5	15	4 54 0 36	35
Parents separated	4	8	10	3	8	3 27 0 29	33
Father deceased	4	10	6	6	3	2 83 0 24	29
Total	96	464	401	194	152		1 307

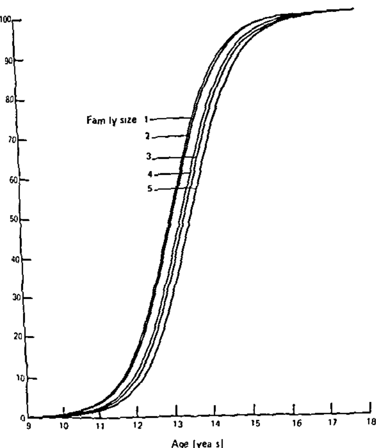


Fig 1 Effect of family size on menarcheal age (Northumberland data)

groups of girls over 16 since for others in the sample it was necessary to make allowance for the fact that the younger age groups included a greater proportion of girls who were still immature and hence that the recalled dates related preponderantly to the more advanced girls This difficulty however could be overcome by assuming on the basis of the good fit of the logit curves above logistic distributions of menarcheal age Maximum likelihood estimates of the two parameters of the distribution were found for each family size and social class and from these the median ages were estimated These are given in Table 7 for each group in which the number of post menarcheal girls was 5 or more and these estimates are quite similar to those from the logit analysis (Table 6) The overall estimate of median age was 13 30 with an estimated 95% range of 10 92 to 15 67 years again very close These figures indicate that on average the girls neither under nor overestimated their age at menarche

Menarche and physique

To investigate the effect of menarche and the environmental variables on height weight and ponderal index the girls were divided into yearly age groups within which separate multiple regression analyses were performed of each of the physique variables in turn on 0 1 variables representing schools social class family size family position and menarcheal state

In the 131 girls under 11 years of age of whom only one had reached menarche and the 93 girls over 16 all post menarcheal physique could not be related to menarcheal state In the age groups 12–12 99 13– and 14– the regressions of all three physique variables

Table 5 *Main analysis—values of deviance*

	Deviance (χ^2)	d f	*p*
Main effects model	328 6	493	
Increase due to constraining hypotheses			
No family size effect	10 3	4	<0 05
No family position effect	0 5	2	
No family size or position effect	14 3	6	<0 05
No social class effect	10 7	7	
Effect of age only	24 3	13	<0 05

significance at the 5% level but there appeared to be no differences between schools when the other factors had been allowed for

Main analysis

As these exploratory results had shown no differences between schools for the main analysis all 13 schools including the juniors were combined and the girls grouped according to age, family position and social class The three social class gradings indicating domestic hardship were also incorporated The results are given in Table 5 By comparison with the deviance in the main effects model the increases due to the constraining hypotheses showed the following There is a significant effect of family size (χ^2 10 3 d f 4) which is very largely attributable to a linear component (χ^2 6 9 1 d f $p<0$ 01) This substantially explains the increased deviance produced by deleting both family size and family position jointly from the model and it appears that there is no significant effect of family position per se on age at menarche in this material The effect of social class is not significant

The median age at menarche by family size and social class as calculated from this main analysis is set out in Table 6 The estimates illustrate the general trend to later menarche with increased family size (Fig 1) the mean increment per additional sib being 0 15±0 06 years The absence of any such trend over social classes is not attributable to the low numbers in classes 1 and 5 The lowest estimated median age is for social class 5 but this is not significantly different from the median age for class 1 or from that for class 4 The overall estimate of median age at menarche for the Northumberland sample as a whole is 13 31 years ±0 03 The age limits between which 95% of girls attain menarche are estimated to be 10 91±0 13 and 15 71±0 13 years

Validity of recall data

At the time of interview 637 of the girls had attained menarche and the month and year of onset as recalled were recorded from all but 2 From these recall data their ages at menarche could be calculated Mean age at menarche could only be found simply for

Table 6 *Median age at menarche according to family size and social class as estimated by main logit analysis*

	Family size					
	1	2	3	4	5+	Total
Social class						
1	13 28	13 32	13 67	13 57	13 84	13 49
2	13 07	13 11	13 46	13 36	13 63	13 [illegible]9
3	13 07	13 11	13 46	13 36	13 63	13 [illegible]7
4	13 41	13 45	13 80	13 70	13 97	13 68
5	12 66	12 70	13 05	12 95	13 22	12 95
Father unemployed	12 74	12 78	13 13	13 03	13 30	13 11
Parents separated	12 90	12 94	13 29	13 19	13 46	13 19
Father deceased	13 00	13 04	13 39	13 [illegible]9	13 56	13 23
Total	13 06	13 13	13 47	13 37	13 62	*13 31*

difference of the proportions inf domestic hardship It appears that the first objective of the sampling has been obtained However the two samples differ in family size The mean in the Northumberland sample is 2 99 s d 1 42 and in the South Shields sample 3 61 s d 2 09 This difference would be expected to act towards earlier menarche in the Northumberland sample and thus reinforce any difference that may be attributable to socioeconomic differences between the two localities

The Northumberland menarcheal age results are very similar indeed to those of the South Shields survey not only in the overall medians (South Shields 13 43±0 05 Northumberland 13 31±0 03) but also in the medians for each family size Thus for families of increasing size from 1 to 5+ the South Shields results of 13 04 13 14 13 45 13 68 and 13 66 are very similar to the Northumberland figures of 13 06 13 13 13 47 13 37 and 13 62 respectively the main difference still slight is that family size 4 which in South Shields was almost identical in median with family size 5 has in Northumberland developed an earlier median menarcheal age more similar to family size 3 This may be due to mere chance Otherwise if the family size effect within a sample is brought about through concealed poverty it suggests either that a greater proportion of the better-off and fewer other parents in south east Northumberland choose a family size of 4 or that families of size 4 are at some critical point responsive to the relatively slight advantages of the families in south east Northumberland as compared with those across the river in South Shields Certainly the similarity for families of other given sizes suggests little response to the socioeconomic difference between the two localities The difference between the two overall medians is almost entirely attributable to the different distributions of family size in the two samples and this family size 4 effect

The essential similarity between the South Shields and Northumberland samples effectively disposes of the suggestion that the delay in menarcheal age of the former by one third of a year by comparison with the last reported status quo samples for Britain is to be attributed to economic hardship and environmental retardation of the South Shields industrial area The elevated medians in both samples however still do not establish reversal in the secular trend of menarcheal age It may be that the north eastern populations did not participate in the downward trend to the same extent as elsewhere in Britain for which in the absence of earlier data there is no evidence Secondly they may be genetically distinct from elsewhere in Britain Certainly there is evidence that the north-eastern populations differ in the frequencies of some Mendelian characters (e g ABO blood groups (3) adenosine deaminase (7) adenylate kinase (5)) Since the population of the north east is differentiated from that of the south by their gene frequencies in these Mendelian characters it would not be surprising if there was also some differentiation in the frequencies of genes controlling growth and development and such genetic difference would preclude direct comparison of developmental features

Certainly the present results endorse the absence of a social class effect on menarcheal age parallel to the findings of the South Shields and Swansea surveys (6) and compatible with other studies in Britain (2 4) The family size effect again resembles that observed in Swansea and South Shields not only in its occurrence but also in its magnitude and may well represent a direct environmental effect concealed poverty acting through nutrition or standards of care The absence of a significant family position effect as in South Shields but different from the Swansea material may perhaps be due to the present mode of analysis in which by reason of subsample numbers family position is compressed into three subcategories for the regression coefficients by position within family size give some suggestion that the youngest sib in families of 4

Table 7 *Median age at menarche according to family size and social class as estimated from recalled ages*

	Family size					
	1	2	3	4	5+	Total
Social class						
1	–	13 31	–	–	–	13 36
2	13 14	13 30	13 35	13 11	13 35	13 28
3	13 28	13 11	13 39	13 29	13 54	13 27
4	13 11	13 50	13 59	13 59	14 11	13 61
5	–	12 75	13 30	13 64	13 52	13 24
Father unemployed	–	–	–	–	13 10	13 20
Parents separated	–	12 74	–	–	13 58	13 19
Father deceased	–	12 66	–	13 28	–	13 12
Total	13 14	13 16	13 40	13 31	13 59	13 30

were significant due wholly or partly to the effect of menarcheal status ($p<0\ 001$ except for ponderal index in age group 13– and height in age group 14– for both of which $p<0\ 01$) In the 11– and 15– age groups weight and ponderal index but not height showed significant regressions on menarcheal status ($p<0\ 05$ for both in the older age group $<0\ 01$ in the younger which also showed a family size effect on weight ($p<0\ 05$)) To the overall regression of ponderal index in the age group 12– significant contributions were made by differences between cschools and between family sizes (both $p<0\ 05$) as well as menarcheal status School and family size differences also exerted a significant effect on height in the 14– age group ($p<0\ 01$ and $<0\ 05$ respectively) The effects of these two environmental variables on physique were not found in the other age groups and in view of the large number of regressions examined must be accepted with caution But in no age group did social class or family position make any significant contribution to any physique regression Only menarcheal status showed a consistent effect in each case height and weight being greater for girls who had attained menarche (Table 8) and ponderal index less This appears to be as far as the present cross sectional data will allow analysis for the interrelations of age at menarche, physique and the environment in which girls develop are complex A longitudinal study would be required to distinguish whether the increment here shown indicates a sudden spurt in size at menarche or earlier maturation in girls who are bigger at each age

DISCUSSION

The Northumberland sample was chosen to provide information on a group of girls from the same geographical region as the South Shields sample but in whom there was less likely to be environmental retardation of maturation The socio economic difference between the Northumberland and South Shields samples is illustrated by the distribution of the father s occupation according to the Registrar General s classification The Northumberland sample shows a clear excess of the proportion of categories 1 2 and 3 South Shields of categories 4 and 5 there is little

Table 8 *Estimated effects of menarche on height and weight*

			Increase associated with menarche in	
Age group	Number of girls	Number menstruating	Height (inches)	Weight (lb)
11–12	116	5	1 7±1 2	22 4±7 5
12–13	288	70	2 4±0 4	20 0±2 5
13–14	260	151	1 9±0 3	15 3±2 1
14–15	257	215	1 3±0 4	17 [illegible]±3 2
15–16	16[illegible]	156	0 7±1 1	20 2±8 8

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THE LONG TERM EFFECTS OF PROTEIN ENERGY MALNUTRITION IN EARLY CHILDHOOD ON BONE AGE BONE CORTICAL THICKNESS AND HEIGHT

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From the Royal Infirmary Gloucester Great Britain the Africa Study Centre Leyden Netherlands and the Social Paediatric and Obstetric Research Unit Glasgow Scotland

ABSTRACT Briers P J Hoorweg J C and Stanfield J P (Royal Infirmary Gloucester Great Britain Africa Study Centre Netherlands Social Paediatric and Obstetric Research Unit Glasgow Scotland) The long term effects of protein energy malnutrition in early childhood on bone age bone cortical thickness and height Acta Paediatr Scand 64 853 1975.—Three groups of Ugandan children 18 in each group and one comparison group of 18 children were examined at 11–17 years of age The three groups had previously been ad mitted for treatment of protein energy malnutrition between the ages of 8 to 15 16 to 21 and 22 to 27 months respectively The comparison group had not been clinically malnour ished throughout the period up to 27 months of age The children came from one tribe and from similar socio-economic background and were individually matched on age and sex The bone age was estimated by hand wrist radiography scored for maturity by the Tanner & Whitehouse method The metacarpal index a ratio derived from the medul lary width and full diameter of the mid point of the second metacarpal was used as a meas ure of bone cortical thickness The three malnourished groups are significantly shorter in height than the comparison group but are not different in bone age and metacarpal index No differences are observed between the three groups of children who had been admitted for protein energy malnutrition at different ages The findings are discussed as they relate to the existing literature

KEY WORDS Protein energy malnutrition bone age bone cortical thickness height

Protein energy malnutrition of early childhood is known to retard growth in height and bone age but its long term effects are less certain Full rehabilitation following a period of malnutrition would require complete catch up skeletal growth and maturation such as would achieve the individual s genetically de termined trajectory It has been reported for nutritional disorders other than protein energy malnutrition that this process of catch up may take up to 3 or 4 years depending on the length and severity of the period of nutritional distortion and the adequacy of the rehabilit tion (3 16)

In animal studies however McCance & Widdowson (12) have demonstrated critical periods in growth during which an induced nutritional growth falter gave rise to perma nent deficit in size however adequate the sub sequent diet Furthermore protein energy malnutrition as it affects the infant and young child in developing countries is far from re sembling the closely monitored and controlled process reported in studies such as have been quoted above The episode of clinical malnutrition may have a fairly acute onset precipitated by infection but this is very often preceded or followed by periods of more chronic malnutrition interspersed with further infection Freedom from infection and dietary

or 5+ may be accelerated by comparison with the oldest and middle children both in the South Shields and the Northumberland samples

As regards physique the consistent association of menarcheal status with increased height and increased weight is similar to that in the South Shields data though the amount of the increase in both is slightly less in the Northumberland data except for the 12 year old age group The occurrence of a family size effect on one of the physique variables in three age groups is rather stronger than in South Shields where it emerged only in one However this slight effect and indeed the absence of effect of any other environmental variables expected from other surveys of physique in relation to environment may well be due to the small numbers that are left for analysis when the total sample is broken down by age groups and menarcheal status so that the numbers become insufficient to show any significant effect

In short these findings like those of the South Shields and Swansea investigations suggest that environment in particular standards of nutrition and general care is still an important determinant of age at menarche but that today in Britain it operates primarily through family size and no longer through formerly accepted socio economic categories The Registrar General s categories are today it seems a less effective measure than formerly of differences in housing standards expenditure on food and other variables of potential biological relevance To these instead, family size appears to be a much more sensitive indicator so that poverty that is effective in influencing biological development should perhaps be sought amongst those of large families instead of those of the lower socio economic categories Again however one cannot exclude the possibility that some other factor not considered in this study e g some genetic influence, may be affecting families of different sizes by different amounts

But certainly the findings in the present sample have achieved their object to establish the validity of the estimate for current menarcheal age described in the previous sample from the north east It remains to be seen whether menarcheal age in these areas now remains steady and whether other regions of the country particularly where there is some genetic or environmental differentiation (e g Scotland South West England) show similar menarcheal age relationships

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REFERENCES

1 Dann T C & Roberts D F End of the trend? A 12 year study of age at menarche *Br Med J III* 265 1973 ✓
2 Douglas J W B *The home and the school* MacGibbon & Kee London 1964
3 Kopec A C *The distribution of the blood groups in the United Kingdom* Oxford University Press 1970
4 Nisbet J D & Illesley R The influences of early puberty on test performance at age 11 *Br J Educ Psychol 33* 169 1963
5 Papiha S S Isoenzyme variation in Northumberland *In* D F Roberts & E Sunderland (eds) *Genetic variation in Britain* Taylor & Francis London 1973
6 Roberts D F & Dann T C Influence on menarcheal age in girls in a Welsh College *Br J Prev Soc Med 21* 170 1967
7 Roberts D F & Papiha S S Adenosine deaminase polymorphism in Northern England *Humangenetik 15* 279 1972
8 Roberts D F Rozner L M & Swan A V Age at menarche physique and environment in industrial North East England *Acta Paediatr Scand 60* 158 1971
9 Tanner J M *Growth at adolescence* Blackwell Oxford 1962 2nd edition

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KEY WORDS *Protein energy malnutrition bone age bone cortical thickness height*

Protein energy malnutrition of early childhood is known to retard growth in height and bone age but its long term effects are less certain Full rehabilitation following a period of malnutrition would require complete catch up skeletal growth and maturation such as would achieve the individual s genetically determined trajectory It has been reported for nutritional disorders other than protein energy malnutrition that this process of catch up may take up to 3 or 4 years depending on the length and severity of the period of nutritional distortion and the adequacy of the rehabilitation (3 16)

In animal studies however McCance & Widdowson (12) have demonstrated critical periods in growth during which an induced nutritional growth falter gave rise to permanent deficit in size however adequate the subsequent diet Furthermore protein energy malnutrition as it affects the infant and young child in developing countries is far from resembling the closely monitored and controlled process reported in studies such as have been quoted above The episode of clinical malnutrition may have a fairly acute onset precipitated by infection but this is very often preceded or followed by periods of more chronic malnutrition interspersed with further infection Freedom from infection and dietary

Table 1 *Composition of groups*

	Malnourished children admitted between			Com parison group
	8–15 months	16–21 months	22–27 months	
Boys	V=10	N=10	V=10	V=10
Girls	N=8	N=8	N=8	N=8
Present age mean (yrs)	14 1	14 0	13 9	13 8
Age range (yrs)	12 3–16 8	11 7–16 8	11 7–16 6	11 8–16 7

int ike during the periods of rch ibilit ition may never be enough for the child to reg un his genetic growth tr yectory

Studies of this complex process have pro duced conflicting results Krueger (11) and MacWilliams & De in (14) suggest that height and bone age are adversely affected for a con siderable time after m ilnutrition in inf incy Keet et al (10) report no significant differ ences between former kw ishiorkor p itients and their siblings in height and bone age after 10 years of follow up Garrow & Pike (7) found no evidence of stunting of growth inste id they found that successfully rehabilitated children tended to outgrow their siblings on their return home

Bone cortical thickness has been studied by Blanco et al (4) in a large group of rural Guatem il in children up to 7 ye irs old They showed that the mean bone cortical me isure ments of these children were less than those of American children of the same age and con cluded that these differences were the result of malnutrition

In an attempt to clarify the situation a study was carried out with 11 to 17 year old Ugandan children for whom records of early childhood existed at the British Medical Research Council s Child Nutrition Unit in Kampala Uganda These records concerned both children admitted for protein energy malnutri tion and children who had maintained a reas onably normal nutritional state in their first three years of life and who had attended a rural child clinic also staffed by the Research Unit This communication presents the heights of the children and the findings obtained with hand wrist radiographs The results of other anthropometric measurements and intellectual abilities are to be published

METHOD

Three groups of 18 children who had been admitted for protein energy malnutrition between 8–15 16–21 or 22–27 months of age and who could be traced were selected The mean age at admission for the three groups was 12 7 19 1 and 24 4 months respectively A comparison group of 18 children was selected from the children who had attended the rural clinic and who had not suffered from protein energy malnutrition during early childhood These children had been seen for at least 2 years starting before the age of 12 months and during this period no clinical signs of malnutrition had been recorded and their weights had not fallen below the tenth percentile of the Boston standard (18) All the children during early childhood had lived in the same or similar rural area

A randomized block design was used in which the four groups of children were individually matched on sex and age while only children from one tribe the Baganda were included The groups came from a similar socio-economic background In each group there were 10 boys and 8 girls the mean age at present was nearly 14 years with a range from 11 7 to 16 8 years (Table 1) The malnourished children had been admitted between 9 and 15 years previ ously

The medical data recorded for the malnourished children at admission was submitted to principal compo nent analysis and varimax rotation of seven medical indi cators recorded during admission weight (per cent for age) haemoglobin level serum protein level weight lost after admission the degree of oedema the degree of skin changes and an overall estimate of severity recorded by the attendant doctor A full discussion of this analysis is to be published The analysis resulted in two statistically independent components of malnutrition One was acute malnutrition reflected in the severity of metabolic abnormalities oedema and skin changes the other was chronic undernutrition composed of weight deficit and haemoglobin deficit both reflecting the chronicity of un dernutrition underlying the acute episode For each child both the severity of acute malnutrition and the severity of chronic undernutrition during admission were calcu lated

In the current examination a hand wrist radiograph was obtained for each child and the bone age assessed by the method of Tanner & Whitehouse (19) The maturity of each child was expressed as the delay of the child s bone age behind its chronological age

The metacarpal index originally described by Barnett & Nordin (2) was used as an indicator of cortical thick ness The overall diameter at right angles to the long axis of the mid point of the second metacarpal was measured by dividers and a Vernier rule scale utilising an Elema Schonander binocular magnifier The medullary width at the same point was measured and from these figures the metacarpal index was calculated

Table 2 *Means of bone age delay metacarpal index and height for three malnourished groups and comparison group*

	Malnourished children admitted between			Comparison group
	8–15 months	16–21 months	22–27 months	
Lag in bone age mean (yrs)	0 63	0 77	0 71	0 22
Metacarpal index	46 70	47 80	40 10	48 70
Height (% for age)	9 10	91 80	90 70	95 50

The hand wrist films were examined in random order by one observer who was not aware of the group from which they derived The bone age and metacarpal index were calculated on three separate occasions and the mean results utilised

The height of each child was recorded by the resident nutritionist and is presented as a percentage of the height expected for the child s age according to the Tanner & Whitehouse standards (20)

RESULTS

The means of bone age delay metacarpal index and height for each group are presented in Table 2

Malnourished children vs Comparison children (analysis of variance randomized block design)

This analysis shows that height is significantly shorter in the previously malnourished children ($F=15\ 5$ $df=1\ 5$) $p<0\ 01$) Bone age delay and metacarpal index are not significantly different ($F=2\ 40$ $F=0\ 0$) although the malnourished children show a relative delay in bone age of half a year

Table 3 *Correlations of acute malnutrition and chronic undernutrition with bone age delay metacarpal index and height (malnourished groups pooled $N=3\times18$)*

	Acute malnutrition	Chronic under nutrition
Bone age lag	−0 19	0 09
Metacarpal index	−0 19	0 03
Height (% for age)	0 12	−0 8

$p<0\ 05$ one tailed test

Children admitted early (8–15 months) vs Children admitted later (16–21 and 22–27 months)

Analysis of variance shows that no significant differences result from the age at which the malnourished children were admitted to the clinic either on bone age delay metacarpal index or height

Acute malnutrition and Chronic undernutrition

As described above the malnourished children had been scored for the severity of these two components of malnutrition during their admission Of the two the first shows no significant correlations with the three dependent variables (Table 3)

Chronic undernutrition correlates negatively with present height while the correlations with bone age delay and metacarpal index are small This agrees with the previous results and confirms that in the long run only height is significantly affected as distinct from bone age delay and metacarpal index

Catch up between 12 and 16 years of age

The correlation of present age with height (percentage for age) is 0 02 among the malnourished children If these children were catching up through prolonged growth their height (per cent for age) would improve with age and a positive correlation between the two would therefore be expected This is clearly not the case and indicates not only that height is permanently affected but also that no catch up has occurred in this age group

Similarly present age and bone age delay correlate −0 02 among the malnourished children indicating that no compensation of previous arrears occurs Table 4 shows this in a different form by presenting the results for two different age groups

Table 4 *Means of bone age delay metacarpal index and height for two groups of malnourished children of different age*

	Malnourished children present age between		Comparison group
	11 7–13 6 yrs	13 8–16 8 yrs	
Number of children	N=26	N=28	N=18
Present age mean (yrs)	12 9	15 0	13 8
Bone age lag mean (yrs)	0 73	0 66	0 22
Metacarpal index	47 6	48 1	48 2
Height (% for age)	91 4	91 7	95 5

No differences appear between the youngest and the eldest age group

Sex boys vs girls

A breakdown of the results for boys and for girls is presented in Table 5 Although bone age appears slightly more delayed among boys than among girls this applies to the comparison children as well The minor tendency of a relatively greater delay in bone age to occur with malnutrition among boys is not corroborated by the height findings which are virtually identical for boys and girls The small differences in the metacarpal index appear erratic The analysis of variance in the previous sections 1 and 2 also revealed no significant interaction effects for sex on any of the three dependent variables

DISCUSSION

The results obtained from this study indicate that protein energy malnutrition in infancy affects height but has no demonstrable long term effect on bone age or metacarpal index when measured 9–15 years later The height deficit is related to the severity of chronic undernutrition in infancy but is not related to the severity of acute malnutrition nor to the age period during which the acute attack required hospitalisation occurred There are no indications that any catch up in bone growth occurs between 12 and 16 years of age The height findings parallel the differences found between heights and weights of children from poor resource countries and children from developed countries Habicht et al (9) have emphasized that these differences are largely due to environmental constraints on growth and development and not to ethnic factors

The present study finds not only that bone age and cortical thickness are not significantly affected by malnutrition at ages 12 to 16 but also that this applies equally to children who suffered an episode of acute malnutrition at different ages No indications are found that any compensation has occurred within the present age group Many of the children in the previously malnourished group had a bone age assessment recorded at the time of admission Unfortunately only a few of these records could be found they all indicated definite retardation of bone age Assuming that bone age was retarded in early childhood one of two conclusions can be drawn The early retardation as a result of protein energy malnutrition was either too small to be still significant at later ages or a catch up has occurred before 12 years of age

These findings are in agreement with the catch up of bone age and metacarpal index found by Barr et al (3) in treated coeliac disease They seem however at variance with previous studies from East Africa Mackay (13) in a group of normal East African children demonstrated a bone age delay of 1½ to 2 years compared with the American

Table 5 *Means of bone age delay metacarpal index and height for boys and girls*

	Malnourished children		Comparison group	
	boys	girls	boys	girls
Number of children	N=30	N=24	N=10	N=8
Lag in bone age mean (yrs)	1 01	0 30	0 40	−0 03
Metacarpal index	46 10	50 00	43 60	53 90
Height (% for age)	91 30	91 80	95 50	95 40

standards In Kampala Krueger (11) assessed a group of children who had been malnourished 6–11 years previously and compared their bone age with that of a group of American children who form the basis of the standard atlas of Greulich & Pyle (8) She reported that bone ages were one or two years below those to be expected from the children s chronological age but drew no further conclusions from this observation

Since her work however studies have been published showing variation in bone age between the American standards of Greulich & Pyle and other groups of children from Sweden (1) Melbourne (17) Hong Kong (5) and Dakar (15) These variations may reflect differences in developmental maturity but also result from different methods of assessment This has been clearly demonstrated by Fry (6) who applied the Tanner & Whitehouse method of assessment of bone age to the plates in the Greulich & Pyle atlas and found a mean overall advance in the Tanner & Whitehouse estimation of over 16 months with a range between −4 1 and +40 4 months This may explain why in the present study which used the Tanner & Whitehouse method the delay of the malnourished children was only 0 7 year with the standards while Krueger & Mackay using the Greulich & Pyle standards reported an age delay of 1½ to 2 years

Krueger s observations are difficult to relate to the presence of malnutrition in the absence of a comparison group of Ugandan children who had not suffered from protein energy malnutrition This is also the case with the study of cortical thickness by Blanco et al (4) Their conclusion that the difference in mean bone cortical measurements between Guatemalan and American children was the result of malnutrition may be true The effect of malnutrition on the cortical index could be better assessed by comparison within a single defined population with a documented history of nutritional status rather than by comparison of different populations

The current findings appear to parallel those of the comprehensive study by Keet et al (10) of 123 children who had suffered from kwashiorkor and were subsequently followed for 10 years These authors used the Greulich & Pyle standards and found no differences in bone age between the previous malnourished children and their siblings Neither however did the two groups of children differ in height throughout the 10 year study This may well indicate that although the siblings differed in not having experienced an acute episode of protein energy malnutrition both groups may have suffered similar degrees of chronic undernutrition The present study however demonstrates that although height has been affected by chronic undernutrition in early childhood no significant ultimate differences in bone age and bone cortical thickness are present

Keet et al and Blanco et al report that independent of malnutrition boys lag further behind American standards in bone age and cortical thickness than girls This study finds a similar though minor trend for bone age but not for cortical thickness and height

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REFERENCES

1 Anderson E Comparison of Tanner Whitehouse and Greulich Pyle methods in a large Swedish survey *Am J Phys Anthropol 35* 373 1971

2 Barnett E & Nordin B E C *The radiological diagnosis of osteoporosis a new approach* *Clin Radiol 11* 166 1960

3 Barr D G D Schmerling D H & Prader A Catch up growth in malnutrition studied in coeliac disease after institution of gluten free diet *Paedi tr Res 6* 521 1972

4 Blanco R A Acheson R M Canosa C & Salomon J B Sex differences in retardation of skeletal development in rural Guatemala *Pediatrics 50* 912 1972

5 Fry E I Tanner Whitehouse and Greulich Pyle Skeletal Age Velocity Comparisons *Am J Phys Anthropol 35* 377 1971

Table 4 *Means of bone age delay metacarpal index and height for two groups of malnourished children of different age*

	Malnourished children present age between		Comparison group
	11 7–13 6 yrs	13 8–16 8 yrs	
Number of children	N = 26	N = 28	N = 18
Present age mean (yrs)	12 9	15 0	13 8
Bone age lag mean (yrs)	0 73	0 66	0 22
Metacarpal index	47 6	48 1	48 2
Height (% for age)	91 4	91 7	95 5

No differences appear between the youngest and the eldest age group

Sex boys vs girls

A breakdown of the results for boys and for girls is presented in Table 5 Although bone age appears slightly more delayed among boys than among girls this applies to the comparison children as well The minor tendency of a relatively greater delay in bone age to occur with malnutrition among boys is not corroborated by the height findings which are virtually identical for boys and girls The small differences in the metacarpal index appear erratic The analysis of variance in the previous sections 1 and 2 also revealed no significant interaction effects for sex on any of the three dependent variables

DISCUSSION

The results obtained from this study indicate that protein energy malnutrition in infancy affects height but has no demonstrable long term effect on bone age or metacarpal index when measured 9–15 years later The height deficit is related to the severity of chronic undernutrition in infancy but is not related to the severity of acute malnutrition nor to the age period during which the acute attack requiring hospitalisation occurred There are no indications that any catch up in bone growth occurs between 12 and 16 years of age The height findings parallel the differences found between heights and weights of children from poor resource countries and children from developed countries Habicht et al (9) have emphasized that these differences are largely due to environmental constraints on growth and development and not to ethnic factors

The present study finds not only that bone age and cortical thickness are not significantly affected by malnutrition at ages 12 to 16 but also that this applies equally to children who suffered an episode of acute malnutrition at different ages No indications are found that any compensation has occurred within the present age group Many of the children in the previously malnourished group had a bone age assessment recorded at the time of admission Unfortunately only a few of these records could be found they all indicated definite retardation of bone age Assuming that bone age was retarded in early childhood one of two conclusions can be drawn The early retardation as a result of protein energy malnutrition was either too small to be still significant at later ages or a catch up has occurred before 12 years of age

These findings are in agreement with the catch up of bone age and metacarpal index found by Barr et al (3) in treated coeliac disease They seem however at variance with previous studies from East Africa Mackay (13) in a group of normal East African children demonstrated a bone age delay of 1½ to 2 years compared with the American

Table 5 *Means of bone age delay metacarpal index and height for boys and girls*

	Malnourished children		Comparison group	
	boys	girls	boys	girls
Number of children	N = 30	N = 24	N = 10	N = 8
Lag in bone age mean (yrs)	1 01	0 30	0 40	−0 03
Metacarpal index	46 10	50 00	43 60	53 90
Height (% for age)	91 30	91 80	95 50	95 40

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NEUROLOGICAL INVESTIGATION OF 5 YEAR OLD CHILDREN WITH LOW BIRTHWEIGHT

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ABSTRACT Bjerre I (Department of Paediatrics Malmo General Hospital Malmo Sweden) Neurological investigation of 5 year-old children with low birthweight Acta Paediatr Scand 64 859 1975.—139 children with a low birthweight (LBW) i e not more than 2 500 g were examined at 5 years of age in respect of their neurological status with special reference to motor coordination according to Touwen & Prechtl 5 (3.6%) children had cerebral palsy 13 (9.4%) minimal brain dysfunction 38 (27.3%) delayed motor maturation and 83 (59.7%) normal motor development and normal neurological status Impairment of hearing and of vision epilepsy and mental retardation were more common in children with cerebral palsy and MBD Children with MBD and delayed motor maturation had a lower IQ as judged from the draw a mantest according to Goodenough than children with normal neurological status Cerebral palsy was found in children with a very low birthweight and short gestational age Other findings were equally distributed among groups classified by weight and duration of gestation

KEY WORDS Low birthweight gestational age cerebral palsy minimal brain dysfunction delayed motor maturation

In recent years the perinatal mortality among infants with a low birthweight (LBW) has been reduced by more intense obstetric and paediatric care (4 12) Infants with LBW are predisposed to diseases of the CNS (5 6) Knowledge of their long term development is important for assessing the value of various forms of treatment given (14) Such appraisal is difficult especially if mild handicaps are to be taken into account as well as several factors capable of influencing development after the neonatal period The present investigation is based on a review at 5 years of age of an unselected well-defined group of children with LBW It aims at assessing the frequency of neurological symptoms and signs in this group as well as the frequency of certain factors associated with the development of a handicap and thereby regarded as risk factors

MATERIAL

The material consisted of 139 children (69 girls and 70 boys) They constituted 90% of all infants with a birth weight ≤2 500 g born in Malmo in 1966 and alive after the first month of life The town has a relatively uniform population the socio-economic standard is good as is the medical service offered All children are born at *one* maternity department and all children requiring treatment are referred to *one* department of paediatrics

Of the 139 children examined 9 weighed not more than 1 500 g at birth 32 between 1 501 and 2 000 g and 98 between 2 001 and 2 500 g 50 infants were born before the end of the 35th week of pregnancy 45 in the 36th–38th week and 44 after the beginning of the 39th week (only one of these after the end of the 42nd week) 52 (37.4%) of the infants were appropriate for gestational age (AGA) according to Swedish normal curves for intrauterine growth (15) and 87 (62.6%) of the infants were small for gestational age (SGA) with a birthweight below the 10th percentile 22 (15.8%) of the infants were twins

Neonatal period

All the infants were treated uniformly during the neonatal period The Apgar score was estimated at 1 minute In the

6 Fry E I Assessing skeletal maturity comparison of the Atlas and Individual Bone techniques *Nature 220* 496 1968
7 Garrow J S & Pike M C The long term prognosis of severe infantile malnutrition *Lancet I* 1 1967
8 Greulich W W & Pyle S I *Radiographic Atlas of Skeletal Development of the Hand and Wrist* Oxford University Press London 1959
9 Habicht J P Martorell R Yarbrough C Malina R M & Klein R E Height and weight standards for pre school children how relevant are ethnic differences in growth potential *Lancet I* 611 1974
10 Keet M P Moodie A D Wittmann W & Hansen J D L Kwashiorkor a prospective ten year follow up study *S Afr Med J 85* 1427 1971
11 Krueger R H Some long term effects of severe malnutrition in early life *Lancet II* 514 1969
12 McCance R A & Widdowson E M Nutrition and growth *Proc R Soc (Series B) 156* 326 1962
13 Mackay D H Skeletal maturation in the hand a study of development in East African children *Trans R Soc Trop Med 46* 135 1952
14 MacWilliam K M & Dean R F A The growth of malnourished children after hospital treatment *East Afr Med J 42* 297 1965
15 Michaut Barthod E *Determination de la maturation osseuse de 277 adolescent Dakarios* Thesis Université de Paris 1971
16 Prader A Tanner J M & von Harnack G A Catch up growth following illness or starvation *J Pediatr 62* 646 1963
17 Roche A F Davila G H & Eyman B S A comparison between Greulich Pyle and Tanner Whitehouse Assessments of Skeletal Maturity *Radiology* 98 273 1971
18 Stuart H S & Stevenson S S *In* W E Nelson (ed) *Textbook of Pediatrics* Saunders Philadelphia 1964 p 48
19 Tanner J M & Whitehouse R H *Standards for skeletal age* International Children s Centre 1959
20 Tanner J M Whitehouse R H & Takaishi M *Standards from birth to maturity for height weight height velocity and weight velocity British children 1965 Arch Dis Child 41* 454 1966

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(I) *Cerebral palsy* (CP) This group contained 5 children with obvious neurological dysfunction i e cases which are easily identifiable without detailed examination as classic cases of cerebral palsy (16) Of the 5 children with CP 4 had spastic diplegia (2 mild 1 moderate and 1 severe) and 1 child had severe dystonic tetraplegia and athetosis In 4 of these children the diagnosis had been made before the age of 1 year and mild spastic diplegia in one child had been diagnosed at 1½ years

(II) *Minimal brain dysfunction* (MBD) In this group are included 13 (9 4%) children The diagnosis was made on the findings of inconspicuous nervous dysfunction i e symptoms revealed only after detailed neurological examination (16) 5 of these 13 children showed a classifiable neurological syndrome Mild *ataxia* in 2 mild choreoathetosis in 2 and mild hemisyndrome in 1 These types of MBD are by some authors classified as minimal cerebral palsy e g by Kong in *Minimal cerebral dysfunction* (1) 8 children were very clumsy and scored pathological or doubtful in most of the tests for fine and gross motor coordination

(III) *Delayed motor maturation* This group consisted of 38 (27%) children in whom the results of two to four of the coordination tests were uncertain or pathological though the children otherwise appeared normal

(IV) *Normal* 83 (60%) children showed no abnormalities as described above

Impairment of hearing was noted in 2 (1 4%) children (Table 1) In both of them the diagnosis had been made at 1 year of age Severe *impairment of vision* was found in 1 child with congenital cataract also diagnosed before the age of 1 year 11 children had strabismus 1 ptosis and 9 mild impairment of vision All children except one with mild impairment of vision not requiring glasses were known before the age of 5 years None of the children had retrolental fibroplasia *Speech retardation* was noted in 28 (20 1%) children and in 13 of them it was severe enough to require special investigation and/or education by a speech therapist Of the 139 children 9 (6 5%) had experienced *convulsions* after the neonatal period 3 of them had been diagnosed as having epilepsy (2 2%) 4 as having benign febrile convulsions and 2 had had one single obscure attack which had received no treatment and had not recurred

Table 2 *IQ according to the draw a mantest compared with neurological findings*

IQ	≤69	70–89	90–109	≥110
CP	0	0	2	1
MBD	1	6 S	4	1
Delayed motor maturation	1	18 S	17	2
Normal	0	20	40	22
Total number	2	44	63	26
per cent	1 4	31 7	45 3	18 7

2 children were not able to draw because of severe CP and mental retardation and from 2 children drawings were not available Low quotients (≤89) were significantly more common in MBD as well as in delayed motor maturation than in normal children The statistical analysis was done with Fisher s exact test

The *IQ according to the draw a man test* and its relation to neurological status is given in Table 2 *Mental retardation* (IQ less than 70) had been diagnosed earlier in 3 children (2 with severe mental retardation diagnosed before the age of 1 year and 1 child with mild mental retardation diagnosed at 3 years) In addition 1 child had an IQ below 70 on the draw a man test He was referred for psychological examination and the level of his mental development was thought to border retardation

The distribution of neurological findings among birthweight groups and degree of maturity of the children at birth is given in Table 3 The 5 children with clear cerebral palsy weighed less than 2 000 g at birth and 4 of them were born before the 35th week of pregnancy and were AGA This means that 12% (95% confidence limits 4–26%) of the children who weighed less than 2 000 g at birth had cerebral palsy and that 8% (95% confidence limits 2–19%) of the children born before the

Table 1 *Sex distribution and distribution of impairment of hearing vision and speech an frequency of convulsions and mental retardation in 139 LBW children at 5 years of age*

	Total	Girls	Boys	Impairment of hearing	Strabismus	Impairment of vision	Impairment of speech	Convulsions	Mental retardation
CP	5	3	2 NS	0	0	2 S	3 S	2 S	2 S
MBD	13	4	9 S	2 S	2 NS	5 S	9 S	1 NS	1 NS
Delayed motor maturation	38	12	26 S	0	3 NS	2 NS	8 NS	3 NS	(1) NS
Normal	83	50	33	0	6	1	8	3	0
Total	139	69	70	2	11	10	28	9	3 (+1)

* Borderline The statistical calculation was done with the chi square analysis in the comparison of the frequency of boys and girls For the other columns it was made with Fisher s exact test *The findings in the groups CP MBD and delayed motor maturation* were separately compared with the findings among LBW-children judged as normal S=significant $p<0.05$ NS=non significant

event of signs of asphyxia the infant was treated with cleaning of the upper airways administration of oxygen by mask and when necessary external heart massage intubation and correction of acidosis All infants with a very low birthweight (≈2000 g) or with respiratory distress were cared for in an incubator with oxygen if indicated No infant was placed in a respirator Feeding was started 6–12 hours after birth with glucose and/or breast milk When it was not possible to feed the infant by mouth 5% glucose was given via a catheter in the umbilical vein (18 infants) 33 infants had neonatal hyperbilirubinaemia (serum bilirubin ≥15 mg/100 ml) Exchange transfusions were given to 7 of these infants usually because of a serum bilirubin exceeding 20 mg/100 ml (Only 1 infant had Rh haemolytic disease) Neither phototherapy nor any other treatment was given to prevent jaundice

METHODS

The children underwent a thorough physical examination at the age of 5 years Most of them had been examined at regular intervals by paediatricians at the children s welfare centre or department of paediatrics Data from these records were registered and completed by interviews with the mothers

Neurological examination was performed in accordance with Touwen & Prechtl (16) but with certain modifications Only the examinations appropriate for children 5 years of age were chosen and the findings were evaluated only according to a three grade scale (normal uncertain abnormal) *Tonus posture and associated movements* were judged with the child sitting standing and walking *The examination of reflexes* comprised the ankle knee biceps and triceps jerks and plantar response Further assessment of *gross movements and their coordination* was made while the child was walking along a straight line standing and hopping on one leg and standing with the arms extended *Hand coordination and small movements* were assessed with the finger–nose test threading of small wooden beads on a string and by drawing

Visual acuity had as a rule been tested at the children s welfare centre with Bostrom s hooks (11) otherwise it was done in association with the neurological examination In all cases with pathological or doubtful findings the children were referred to a specialist

In half of the cases *hearing* had been tested with play audiometry at the children s welfare centre in the other half of the children it was done by whispering at 4 meters as a part of the neurological examination

Speech development was judged as retarded if the child could not say single words at 18 months could not form sentences at 2 5 years or did not speak clearly at 5 years of age

Spontaneous drawing of the human being (draw a man) was judged according to Goodenough (7)

Statistical calculations were done with the chi square analysis or if the expected numbers were too small for an approximative method with Fisher s exact test The confidence limits given are the exact limits for the binomial distribution The level of statistical significance chosen is 5% i e p less than 0 05 is marked S a p value above 0 05 NS

RESULTS

Cooperation at the examination was normal in most cases 3 children had a doubtful cooperation because of anxiety and negativism and in these cases one or two of the subtests had to be omitted 1 child had a Thomas splint because of coxa plana and could therefore not be judged in respect of gait balance and hopping 2 children had mental retardation in association with cerebral paresis so that only gross neurological evaluation was possible

On the basis of the findings in the neurological examination the children were divided into four groups

(I) *Cerebral palsy* (CP) This group contained 5 children with obvious neurological dysfunction i e cases which are easily identifiable without detailed examination as classic cases of cerebral palsy (16) Of the 5 children with CP 4 had spastic diplegia (2 mild 1 moderate and 1 severe) and 1 child had severe dystonic tetraplegia and athetosis In 4 of these children the diagnosis had been made before the age of 1 year and mild spastic diplegia in one child had been diagnosed at 1½ years

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Table 3 *Children with neurological disease and delayed motor maturation grouped according to birthweight and degree of maturity at birth (week of gestation)*

	Week of gestation				
	Birthweight ≤2000 g		Birthweight 2001–2500 g		
	≤35	≥36	≤35	≥36	Total
CP	4 S	1	0	0	5
MBD	4	2	1	6	13
Delayed motor maturation	7	3	8	20	38
Normal	14	6	12	51	83
Total	29	12	21	77	139

Statistical calculations were done with Fisher s exact test In the calculations comparisons were made between the frequency of CP MBD and delayed motor maturation separately for birthweight of at most 2000 g and above 2000 g and for gestational week at most 35 and more than 35 Statistically significant differences were found for children with CP both regarding birthweight and week of gestation In the other groups no statistically significant difference was found for these two parameters

35th week of pregnancy had cerebral palsy Motor development of 49% (95% confidence limits 33–65%) of the children with a birth weight of not more than 2000 g and 64% (95% confidence limits 54–74%) of the children with a birthweight of 2001–2500 g was normal For CP there was a statistically significant pre ponderance of a birthweight of not more than 2000 g and pregnancy of less than 35 weeks As for retarded motor development and neurological signs of doubtful nature no statistically significant difference was found between weight groups and degree of maturi ty

Obstetric and neonatal complications No correlation was found between obstetric com plications such as bleeding toxemia placental infarction or type of delivery and neurological findings at 5 years of age

Neonatal complications (Table 4) were most common in the CP group in which one or more complications occurred in every case In the MBD group only 2 of 13 children had had no complication in the neonatal period In the groups categorized as delayed motor matura tion and normal no neonatal complication oc curred in 54% and 45% respectively A low Apgar score respiratory distress syndrome hyperbilirubinaemia and neonatal convulsions were not more common in MBD and delayed motor maturation than in the normal group

Table 4 *Occurrence of low Apgar score respiratory distress syndrome hyperbilirubinaemia (serum bilirubin ≥15 mg/100 ml) neonatal convulsions and other diseases in the neonatal period in 139 LBW children distributed between 4 groups according to the neurological findings at 5 years of age*

	Uncomplicated neonatal period		Apgar score ≤6	Respiratory distress syndrome	Hyperbili rubinaemia	Neonatal convulsions	Various
	n	%					
CP	0	0	3 S	2 NS	3 NS	1 NS	1 NS
MBD	2 S	15 4	0	5 NS	4 NS	1 NS	4 S
Delayed motor maturation	17 NS	44 7	2 NS	4 NS	9 NS	0	6 NS
Normal	45	54 2	12	13	17	2	4
Total	64	46 0	17	24	33	4	15

A child may have had more than one complication in the neonatal period Therefore the table includes also the number of children judged as having an uncomplicated neonatal course apart from low birthweight The hypothesis that an uncomplicated neonatal period is less common and that each neonatal complication separately is more common in the groups with neurological abnormalities at 5 years of age was tested with Fisher s exact test In statistical analysis the findings in the groups CP MBD and delayed motor maturation were each separately compared with the findings in the group with normal development S=significant $p<0.05$ NS=non significant

The distribution of the neurological deviations on a scale of scores covering social group economy civil status and need of social help (3) showed no demonstrable effect of these social factors on the neurological findings Living in a group and *preschool training* would perhaps result in better coordination and balance But no demonstrable difference in these parameters was found between children living at home and children who took part in various types of preschool at 5 years of age

DISCUSSION

The frequency of CP found in this material agreed largely with figures reported by earlier authors (10) The preponderance of spastic diplegia is also well in accordance with other reports (8 10) In recent years the frequency of spastic diplegia among low birthweight infants has decreased (8 14) This material is not large enough to warrant any such conclusion but though the perinatal mortality may be regarded as low at least nothing suggested that the number of seriously handicapped children had increased

It is also clear from the investigation that all real handicaps were diagnosed early in children followed up at regular intervals at the children s welfare center Therefore at a review at 5 years of age there is no reason to expect the findings of any cases missed at earlier routine examinations (9) Of greater interest at this age is examination of the children for *minor cerebral injuries* Some authors have reported a low developmental quotient and a high incidence of minor injuries in low birth weight children (6 18) while others are more optimistic (2) The difficulty in characterising these minor cerebral lesions is obvious A good survey of *minimal brain dysfunction* is given in a symposium edited by Bax & McKeith (1) Differentiation from psychiatric disease is difficult but Touwen & Prechtl (16) have given a detailed description of the neurological diagnosis Many workers feel that MBD is a syndrome that should be diagnosed in preschool age so that one can start measures to prevent school problems and behaviour disorder This would be a reason for screening all children e g at 4 years of age (9) or for a more careful observation of children in certain risk groups (13) In the present material it was found that 9% of all LBW-children could be diagnosed as MBD (95% confidence limits 5–15%) according to the criteria used This means significantly more MBD children than in Kohler s normal population in which the same examination technique was used and an MBD frequency of 2% was found (9) In the present material as in Kohler s about half of the children classified as MBD also had more notable neurological symptoms the other half was merely defined as consisting of very clumsy children

It is still more difficult to find comparative materials for the delayed motor maturation group Touwen & Prechtl do not give figures for the permissible percentage of deviation from the mean results of various motor coordination tests in a normal population Vuille (17) described a group of 4 year old children examined in a way comparable to the present material He found only 6% with deviations in gross motor coordination

Neither children with delayed motor maturation nor MBD children were found to belong to any special weight group nor to have any particular perinatal complications Boys were however overrepresented in both groups Except for the children with a coexisting handicap such as impairment of hearing or vision they were regarded by their parents as healthy and normally developed Their IQ according to the draw a man test was somewhat lower than that of children with normal motor development But the draw a mantest permits only a rough estimation of mental development and requires some degree of visuo motor maturity which means that it may perhaps measure only a certain retardation of motor maturity Neither was there any evidence suggesting that children with MBD and delayed motor maturation constituted understimulated

groups There was no difference in the frequency of delayed motor maturation in the different social groups and motor maturation also seemed to be independent of whether the children attended a preschool or not

This investigation is part of a longitudinal study It showed that overt CNS disease as a rule was diagnosed long before the age of 5 years At 5 years of age the LBW children showed much poorer motor coordination than expected for age but this immature motor development was not confined to any particular group that could be recognised from perinatal data It is intended to follow up the children during school age and try to find out whether those children who had been found to have minimal brain dysfunction and delayed motor maturation have special problems making it difficult for them to adapt themselves to school life

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I wish to thank psychologist Eva Hansen for assessing the drawings and nurses and doctors at the children s welfare clinics for giving me access to their records

REFERENCES

1 Bax M & MacKeith R (eds) *Minimal cerebral dysfunction* Little Club Clinics in Developmental Medicine No 10 William Heinemann Medical Books Ltd London 1963
2 Babson S G & Kangas J Preschool intelligence of undersized term infants *Am J Dis Child 117* 553 1969
3 Bjerre I & Varendh G A study of some biological and socioeconomic factors in low birth weight *Acta Paediatr Scand* In print 1975
4 Bjerre I & Östberg G Infant mortality *Acta Paediatr Scand 63* 49 1974
5 Caputo D V & Mandell W Consequences of low birth weight *Dev Psychol 3* 363 1970
6 Drillien C M Growth and development in a group of children of very low birth weight *Arch Dis Child 33* 10 1958
7 Goodenough F L *Measurement of intelligence by drawings* World Book Company Chicago 1926
8 Hagberg B Hagberg G & Olow I The changing panorama of cerebral palsy in Sweden 1954-1970 I II *Acta Paediatr Scand 64* 187 1975
9 Kohler L Physical examination of four year-old children *Acta Paediatr Scand 62* 181 1973
10 Mc Donald A *Children of very low birth weight* M E I U Research Monograph No 1 William Heinemann Medical Books Ltd London 1967
11 Nordlow W & Joachimsson S A screening test for visual acuity in four year old children *Acta Ophthalmol 40* 453 1962
12 Rawlings G Reynolds E O R Stewart A & Strang L B Changing prognosis for infants of very low birth weight *Lancet 1* 516 1971
13 Sheridan M D Infants at risk of handicapping conditions *Monthly Bull Minist Health 21* 23? London 1962
14 Stewart A L & Reynolds E O R Improved prognosis for infants of very low birth weight *Pediatrics 54* 723 1974
15 Sterky G Swedish standard curves for intra uterine growth *Pediatrics 46* 7 1970
16 Touwen B C L & Prechtl H F R *The neurological examination of the child with minor nervous dysfunction* Clinics in Developmental Medicine No 38 William Heinemann Medical Books Ltd London 1970
17 Vuille J C Den allmanna halsokontrollen av fyra åringar uppspårande av somatiska avvikelser och psykologiska problem *Lakartidningen 68* 456 1971
18 Wiener G Rider R V Oppel W C Fischer L K & Harper P A Correlates of low birth weight psychological status at six to seven years of age *Pediatrics 35* 434 1965

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SHORT COMMUNICATION

MANNOSIDOSIS FINDINGS IN CULTURED FIBROBLASTS AND URINE

E. VAMOS HURWITZ M TONDEUR R HUMBEL[2] M PHILIPPART[3] P HOSLI[4] and H LOEB

Since the original description of mannosidosis by Ockerman (10) six additional patients with this disease have been reported (1 11 14) and seven cases are to be published (3 7)

We wish to report some findings from cultured fibroblasts and urine obtained from siblings observed by Loeb et al in 1969 (5) According to clinical and ultrastructural data Spranger considered these cases as representing instances of mucolipidosis I (12) However unlike one of the cases of mucolipidosis I reported by Spranger et al (13) cultured fibroblasts from the siblings observed by our group did not present the I cell phenotype Moreover histochemical stainings and ultrastructural studies failed to disclose any storage process (6) Indeed kinetic studies with labelled sulfate showed no abnormal accumulation of this precursor (8) In addition the metabolism of mannosides in cultured fibroblasts was investigated

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Departement de Biologie Moléculaire Institut Pasteur Paris

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Following a 2-day pulse with 1[14]C acetate or U [14]C mannose replicate cultures were extracted at regular intervals up to 6 weeks Extracts were separated into water soluble and lipid fractions and insoluble residue Label incorporation was within normal limits with both precursors Following U[14]C mannose pulse most of the lipid counts were recovered in fatty acids from triacyl glycerols and choline phospholipids The half life of the water soluble fraction was 19 days compared with a normal mean of 11 days Most of the water soluble contents were soluble in trichloracetic acid which might represent the glycopeptid material reported by Tsay et al (15)

Acid hydrolase activities were within normal limits with the remarkable exception of α mannosidase which showed a sharply reduced activity to 5–10% of the normal values when assayed at pH 4 4 with both p nitrophenyl and methylumbelliferyl substrates

Screening for abnormal mannoseoligosaccharides was performed on the urine of one of these siblings by thin layer chromatography on silicagel (4) followed by column chromatography on Sephadex hydrolysis and subsequent identification of mannose (9) The presence of abnormal mannose rich oligosaccharides was clearly demonstrated (Fig 1)

COMMENTS

As emphasized by Autio et al (1) the clinical features of the siblings reported by our group

groups There was no difference in the frequency of delayed motor maturation in the different social groups and motor maturation also seemed to be independent of whether the children attended a preschool or not

This investigation is part of a longitudinal study It showed that overt CNS disease as a rule was diagnosed long before the age of 5 years At 5 years of age the LBW children showed much poorer motor coordination than expected for age but this immature motor development was not confined to any particular group that could be recognised from perinatal data It is intended to follow up the children during school age and try to find out whether those children who had been found to have minimal brain dysfunction and delayed motor maturation have special problems making it difficult for them to adapt themselves to school life

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I wish to thank psychologist Eva Hansen for assessing the drawings and nurses and doctors at the children s welfare clinics for giving me access to their records

REFERENCES

1 Bax M & MacKeith R (eds) *Minimal cerebral dysfunction* Little Club Clinics in Developmental Medicine No 10 William Heinemann Medical Books Ltd London 1963

2 Babson S G & Kangas J Preschool intelligence of undersized term infants *Am J Dis Child 117* 553 1969

3 Bjerre I & Varendh G A study of some biological and socioeconomic factors in low birth weight *Acta Paediatr Scand* In print 1975

4 Bjerre I & Östberg G Infant mortality *Acta Paediatr Scand 63* 49 1974

5 Caputo D V & Mandell W Consequences of low birth weight *Dev Psychol 3* 363 1970

6 Drillien C M Growth and development in a group of children of very low birth weight *Arch Dis Child 33* 10 1958

7 Goodenough F L *Measurement of intelligence by drawings* World Book Company Chicago 1926

8 Hagberg B Hagberg G & Olow I The changing panorama of cerebral palsy in Sweden 1954–1970 I II *Acta Paediatr Scand 64* 187 1975

9 Kohler L Physical examination of four year-old children *Acta Paediatr Scand 62* 181 1973

10 Mc Donald A *Children of very low birth weight* M E I U Research Monograph No 1 William Heinemann Medical Books Ltd London 1967

11 Nordlow W & Joachimsson S A screening test for visual acuity in four year old children *Acta Ophthalmol 40* 453 1962

12 Rawlings G Reynolds E O R Stewart A & Strang L B Changing prognosis for infants of very low birth weight *Lancet I* 516 1971

13 Sheridan M D Infants at risk of handicapping conditions *Monthly Bull Minist Health 21* 232 London 1962

14 Stewart A L & Reynolds E O R Improved prognosis for infants of very low birth weight *Pediatrics 54* 723 1974

15 Sterky G Swedish standard curves for intra uterine growth *Pediatrics 46* 7 1970

16 Touwen B C L & Prechtl H F R *The neurological examination of the child with minor nervous dysfunction* Clinics in Developmental Medicine No 38 William Heinemann Medical Books Ltd London 1970

17 Vuille J C Den allmanna halsokontrollen av fyra åringar uppspårande av somatiska avvikelser och psykologiska problem *Lakartidningen 68* 456 1971

18 Wiener G Rider R V Oppel W C Fischer L K & Harper P A Correlates of low birth weight psychological status at six to seven years of age *Pediatrics 35* 434 1965

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SHORT COMMUNICATION

MANNOSIDOSIS FINDINGS IN CULTURED FIBROBLASTS AND URINE

E VAMOS HURWITZ [1] M TONDEUR R HUMBEL [2] M PHILIPPART [3] P HOSLI [4] and H LOEB [5]

Since the original description of mannosidosis by Ockerman (10) six additional patients with this disease have been reported (1 11 14) and seven cases are to be published (3 7)

We wish to report some findings from cultured fibroblasts and urine obtained from siblings observed by Loeb et al in 1969 (5) According to clinical and ultrastructural data Spranger considered these cases as representing instances of mucolipidosis I (12) However unlike one of the cases of mucolipidosis I reported by Spranger et al (13) cultured fibroblasts from the siblings observed by our group did not present the I cell phenotype Moreover histochemical stainings and ultrastructural studies failed to disclose any storage process (6) Indeed kinetic studies with labelled sulfate showed no abnormal accumulation of this precursor (8) In addition the metabolism of mannosides in cultured fibroblasts was investigated

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Following a 2-day pulse with I C acetate or U [1] C mannose replicate cultures were extracted at regular intervals up to 6 weeks Extracts were separated into water soluble and lipid fractions and insoluble residue Label incorporation was within normal limits with both precursors Following U C mannose pulse most of the lipid counts were recovered in fatty acids from triacyl glycerols and choline phospholipids The half life of the water soluble fraction was 19 days compared with a normal mean of 11 days Most of the water soluble contents were soluble in trichloracetic acid which might represent the glycopeptid material reported by Tsay et al (15)

Acid hydrolase activities were within normal limits with the remarkable exception of α mannosidase which showed a sharply reduced activity to 5–10% of the normal values when assayed at pH 4 4 with both *p* nitrophenyl and methylumbelliferyl substrates

Screening for abnormal mannoseoligosaccharides was performed on the urine of one of these siblings by thin layer chromatography on silicagel (4) followed by column chromatography on Sephadex hydrolysis and subsequent identification of mannose (9) The presence of abnormal mannose rich oligosaccharides was clearly demonstrated (Fig 1)

COMMENTS

As emphasized by Autio et al (1) the clinical features of the siblings reported by our group

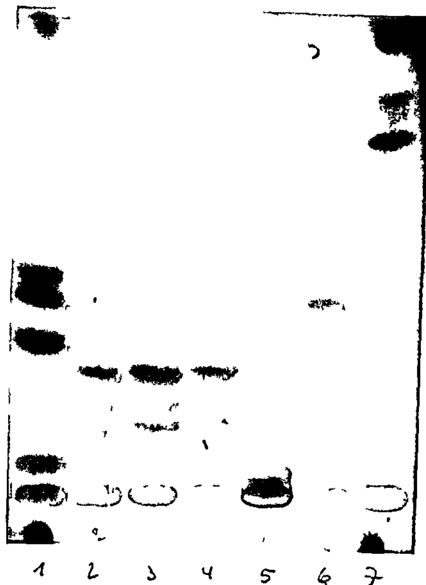


Fig 1 Thin layer chromatography of urinary oligosaccharides Urine from our case (band 3) was compared with specimens from 2 confirmed cases of mannosidosis (bands 2 and 4) note the strong spot of mannose-oligosaccharides Band 1 is urine of a case of aspartylglucosaminuria band 5 of a case of GM_1 gangliosidosis bands 6 and 7 controls

fit rather well those described in the cases of mannosidosis described so far These cardinal signs, which include discrete gargoyle facies impaired hearing and intelligence and vacuolized lymphocytes (2) were all present in our siblings (5)

The ultrastructural findings in the liver are also similar although the electron opaque globules contained in the inclusion were far more numerous in our cases (1 5)

The findings of deficient α mannosidase activity in cultured fibroblasts and of mannose rich oligosaccharides in the urine strongly support in these cases, the suggested diagnosis of mannosidosis

REFERENCES

1 Autio S Norden N E Öckerman P A Riekkinen P Rapola J & Louhimo T Mannosidosis clinical fine structural and biochemical findings in three cases *Acta Paediatr Scand 62* 555 1973

2 Autio S Clinical features of mannosidosis *J Pediatr 86* 314 1975

3 Farriaux J P Legouis I Humbel R Dhondt J L Richard P Strecker G Fourmaintraux A Ringel J & Fontaine G La mannosidose A propos de 5 observations *Nouv Presse Med 4* 1867 1975

4 Humbel R & Collart M Oligosaccharides in urine of patients with glycoprotein storage diseases Rapid detection by TLC *Clin Chem Acta* (in press)

5 Loeb H Tondeur M Toppet M & Cremer N Clinical biochemical and ultrastructural studies of an atypical form of mucopolysaccharidosis *Acta Paediatr Scand 58* 220 1969

6 Loeb H Vamos Hurwitz E Tondeur M & Konyar E Etude ultrastructurale des fibroblastes dans les mucolipidoses *1er Symposium sur les mucopolysaccharidoses* Luxembourg May 6–7 1972
7 Maroteaux P Personal communication
8 Neufeld E F Personal communication
9 Norden N E Lundblad A Svensson S Ockerman P A & Autio S A mannose-containing trisaccharide isolated from urines of three patients with mannosidosis *J Biol Chem 248* 6210 1973
10 Öckerman P A A generalized storage disorder resembling Hurler s syndrome *Lancet II* 239 1967
11 Ormos J Monus Z Szabò L & Csapò Z The significance of liver ultrastructure in the classification of various types of mucopolysaccharidoses *Verh Dtsch Ges Pathol 55* 399 1971
12 Spranger J & Wiedemann H R The genetic mucolipidoses *Humangenetik 9* 113 1970
13 Spranger J Wiedemann H R Tolksdorf M Graucob E & Caesar R Lipomucopolysaccharidosis Eine neue Speicherkrankheit *Z Kinderheilkd 103* 285 1968
14 Tsay G C Dawson G & Matalon R Excretion of mannose rich complexe carbohydrates by a patient with α mannosidase deficiency (mannosidosis) *J Pediatr 84* 865 1974
15 Tsay G C Dawson G & Matalon R Neurovisceral storage disease resulting from α mannosidase deficiency *Trans Am Soc Neurochem 5* 135 1974

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CASE REPORT

GLUCOSEPHOSPHATE ISOMERASE DEFICIENCY IN A DUTCH FAMILY

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ABSTRACT Van Biervliet J P G M (University Children s Hospital Het Wilhelmina Kinderziekenhuis, Utrecht The Netherlands) Glucosephosphate isomerase deficiency in a Dutch family Acta Paediatr Scand 64 868, 1975 —A mentally retarded girl with severe hemolytic anemia due to glucosephosphate isomerase deficiency is described The deficiency was detected in erythrocytes leukocytes thrombocytes liver and muscle tissues Besides the glucosephosphate isomerase deficiency a glutathione instability of unknown origin was found in the erythrocytes of the propositus

KEY WORDS Glucosephosphate isomerase deficiency glutathione instability glucose metabolism hemolytic anemia mental retardation

Glucose 6 phosphate isomerase (EC 5 3 1 9) catalyses the reversible reaction from glucose 6 phosphate to fructose 6 phosphate Since the original description by Baughan et al (4) of glucose 6 phosphate isomerase (GPI) deficiency as a cause of non spherocytic hemolytic anemia other variants of this enzyme have been described (2 3 6 7 10 15 16 18) GPI deficiency is probably the third most frequent cause of enzyme deficient non spherocytic hemolytic anemia after glucose 6 phosphate dehydrogenase deficiency and pyruvate kinase deficiency The mode of inheritance of GPI deficiency is autosomally recessive (4 7 10 11 16 18 21 22 25) A new case of GPI deficiency detected in a mentally retarded girl suffering from hemolytic anemia is described

CASE REPORT

C C an 8 year old girl was admitted to our hospital in May 1974 for treatment of a severe hemolytic disorder She was the second child of parents originating from a small isolated community with a high incidence of consanguineous intermarriage During the last week before birth nitrofurantoin therapy was given to her mother The parents observed jaundice from the first day of life Despite lethargy floppiness and the severe weightloss no medical attention was given in the neonatal period Blood group incompatibility was not demonstrated Psychomotor development was retarded from birth onwards Different intelligence tests showed the same results (IQ ±57) Many hemolytic crises did occur for which she was treated elsewhere Crises were provoked by the frequently occurring severe viral and bacterial infections and also by drugs The cause of the hemolysis was not detected and therapy was symptomatic A therapeutic trial with corticosteroids gave inconclusive results Splenectomy was performed at the age of six Neither the frequency nor the severity of hemolytic episodes diminished The week before admission she received nitrofurantoin in a dose of 5 mg/kg bodyweight for treatment of cystitis There were also persistent complaints of muscular fatigue

Clinical findings

On admission a mentally retarded icteric and anemic girl was seen Some dysmorphic stigmata were obvious turriform skull coarse face synophris hypertelorism epicantus internus folds and yellow coloured teeth The liver was enlarged to 3 cm below the right costal margin There was an S shaped thoraco lumbar scoliosis and all joints were hyperextensible Dysphagia clumsy movements hyperactive deep tendon reflexes muscular hypotonia of the upper and hypertonia of the lower extremities were seen at neurological examination

Table 1 *Activity of glucosephosphateiso merase (in percentage from normal) from different tissues*

	Activity (% from normal)
Erythrocytes	0–?5
Leukocytes	25
Thrombocytes	0–30
Liver	6
Muscle	?8

Laboratory examinations

Methods Microchemical modifications of current blood chemical analyses were used The carbohydrate metabolism was studied as described by Fernandes & van de Kamer (8 9) Vitamin status was assessed by microbiologic methods for B B_2 B $_2$ and folic acid vi tamin E by the method of Quaise et al (?0) Karyotype and urinary and serum aminoacids were examined by standard procedures

Substrates coenzymes and auxiliary enzymes for enzyme and glycolytic intermediates determinations were provided by Bohringer & Sohn All other reagents used were of analytical grade The glycolytic in termediates were determined according to Niessner & Beutler (17) but adenosinetriphosphate according to Minakami et al (14) and ?–3 diphosphoglycerate accord ing to Sigma (?4) Analysis of glutathione and glutathione instability was performed as described by Beutler et al (5) GPI activity was measured according to Slein at 25 C (23) The reduction of NADP was followed at 340 nm with a Perkin Elmer spectrophotometer GPI activity was de termined in 0 1 M Tris HCL pH 8 3 in a final volume of 3 ml containing 3 mM fructose 6-phosphate 0 5 mM NADP 0 01 ml glucose-6-phosphate dehydrogenase and enzyme Homogenization of liver and muscle tissue and leukocytes was carried out in 0 1 M sodium–potas sium–phosphatebuffer pH 7 0 containing 0 1% saponine Leukocytes were obtained by the method of Wyss et al (?7) protein content was determined by the method of Lowry et al (12) with crystalline human serum albumin as a standard

RESULTS

Normal serum values of minerals lipids urea creatinine total protein organic and amino acids were found Acid–base status was nor mal At admission elevation of both unconju gated (55 8 μmol/l) and conjugated (15 7 μmol/l) bilirubin was seen The serum lactate dehydrogenase level in admission was 274 I U and after remission 194 I U its iso enzyme distribution was normal The liver functions serum aldolase and creatine phosphokinase concentrations were normal All renal functions were normal Fehlings reaction was negative There was no aminoaciduria or organic aciduria Ther serum levels of vitamin B_1 B_2 B_{12} folic acid and vitamin E were in the normal range Karyotype was normal (46 XX)

Hemoglobin concentration upon admission was 5 2 mmol/l and decreased within 5 days to 3 3 mmol/l There was a pronounced re ticulocytosis In the blood smears slight hypochromasia polychromasia some sphero cytes and some target cells were seen Serum iron was 18 7 μmol/l total iron binding capac ity was 39 3 μmol/l The osmotic resistance curve was flattened (increased and decreased) No abnormal hemoglobin could be detected

Enzyme and metabolic studies

Besides the decreased GPI activity all other enzyme activities in the erythrocytes were normal or increased In our laboratory the normal values for GPI activities are 28–36 μmoles/min/g Hb In the patient the GPI activity in the erythrocytes was 6 4 μmoles/ min/g Hb which means a reduction to 20–25% of normal

The GPI deficiency was also demonstrated

Table 2 *The content of glycolytic in termediates from normal and the patient s erythrocytes expressed as nanomoles/ml eryth rocytes except for adenosine triphosphate and 2 3 diphosphoglycerate (in μmoles/ml erythrocytes*

	Normal	Patient
glucose-6-phosphate	30– 45	72
fructose-6-phosphate	10– .0	13
glyceraldehyde phosphate	2– 10	2
d hydroxyacetonephosphate	10– .?	11
fructose 1 6-diphosphate	3– 13	3
3 phosphoglycerate	40– 90	44
phosphoenolpyruvate	10– ?4	10
? phosphoglycerate	4– 14	6
pyruvate	50– 00	60
adenosine triphosphate	1 2–1 6	1 2
?3-diphosphoglycerate	5 3±0 4	4 9

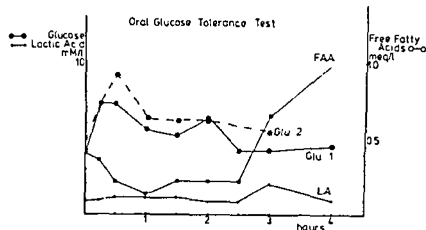


Fig 1 The oral glucose tolerance test (? g glucose per kg bodyweight) The gluco values are within the normal range Th glucose disappearance was followed on second occasion

in leukocytes thrombocytes and in muscle and liver tissues (Table 1) Table 2 shows the content of glycolytic intermediates of the erythrocytes As can be expected in GPI deficiency the glucose 6 phosphate concentration was increased while the other metabolites of the glycolysis showed low normal values

Glutathione metabolism

The glutathione (GSH) concentration in the erythrocytes was normal (4 2 mmol/l erythrocytes) However after administration of 25 mg phenothiazine the GSH concentration fell to 0 7 mmol/l erythrocytes After *in vitro* incubation of erythrocytes with acetylphenylhydrazine according to Beutler (5) GSH content decreased to 1 4 mmol/l erythrocytes which means that a so called GSH instability exists The activity of the enzymes involved in GSH metabolism glucose 6-phosphate dehydrogenase glutathione reductase and glutathione peroxidase showed normal or increased values Also the catalase activity was normal

Carbohydrate metabolism

In order to study the impact of GPI deficiency on the glucose metabolism different oral hexose tolerance tests were performed The glucose tolerance and fructose tolerance tests were within normal limits free fatty acid responses were normal (see Figs 1–2) Since in complete absence of GPI gluconeogenesis is impossible at the step fructose 6 phosphate to glucose 6 phosphate we observed the glucos levels during prolonged fasting After 22 hour fasting blood glucose and lactic acid level were still normal (Fig 3) The lactate forma tion *in vivo* after exercise was normal so wa the oxygen dissociation curve The glycoge content in the liver was high

DISCUSSION

The patient described is suffering from a *generalized GPI deficiency* The enzyme defi ciency was found in erythrocytes thrombo cytes leukocytes hepatocytes and muscle tis sues Arnold et al (1) and Payne et al (19 reported that no tissue specific iso enzymes o GPI exist in man Our observations are in

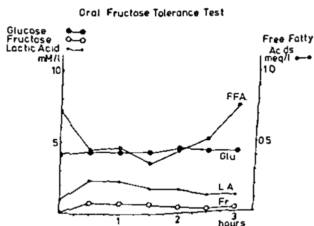


Fig 2 The oral fructose tolerance test (? g fructose per kg bodyweight) Normal values for fructose glucose lactic acid and free fatty acids are seen

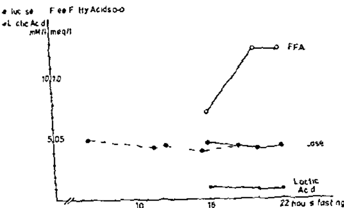


Fig 3 The effect of prolonged fasting on blood glucose levels was observed at first during an 18½ hours fasting period (---) and a second time during a 22 hours fasting period Glucose lactic acid and free fatty acids concentrations are within the normal range The observed GPI-deficiency does not disturb gluconeogenesis

agreement with these references However in contrast to most cases of GPI deficiency reported our patient is mentally retarded It is impossible to evaluate the contribution of the perinatal events to the cerebral damage There were signs of bilirubin intoxication jaundice drowsiness and prolonged icterus Mental retardation without GPI deficiency however is present in other members of the family So a relationship between GPI deficiency and mental retardation is unlikely

In contrast with the literature (18 26) our patient did not benefit from splenectomy Neither the severity nor the frequency of the hemolytic attacks were influenced

Total absence of GPI would severely disturb glycolysis and block gluconeogenesis In GPI deficiency glucose 6 phosphate is increased (see Table 2) One would expect inhibition of hexokinase by glucose 6 phosphate and therefore impaired glucose utilisation As pointed out by Lohr (13) the level of glucose 6-phosphate in GPI deficient patients is not high enough to inhibit hexokinase

Disturbed erythrocytary glycolysis due to hexokinase inhibition is seen only in relatively old erythrocytes (13) In our proband the oral glucose tolerance tests was within normal values and gluconeogenesis was normal since a 22 hour fasting period produced no hypoglycemia and lactic acidemia was absent These data can be explained by the residual activity of GPI

Frequent hemolytic crises are observed in our patient They are provoked by viral and bacterial infections An increased susceptibility to infections is present It is not clear whether there exists a relationship between the observed GPI deficiency or the GSH instability and the increased susceptibility to infections

The cause of the GSH instability is obscure No enzyme deficiencies of the hexose monophosphate shunt are found Studies on granulocyte and lymphocyte functions in relation to the observed increased susceptiblity to infection are in progress

ACKNOWLEDGEMENTS

I am deeply indepted to Dr G Staal and Dr P Helleman for the hematologic examinations

REFERENCES

1 Arnold H Blume K G Busch D Lenkeit U Lohr G W & Lubs E Klinische und biochemische Untersuchungen zur Glucosephosphatisomerase normaler menschlicher Erythrocyten und bei Glucosephosphatisomerasemangel *Klin Wochenschr* 21 1 99 1970

Arnold J Blume K G Engelhardt R & Lohr G W Glucosephosphate isomerase deficiency evidence for in vivo instability of an enzyme variant with hemolysis *Blood* 41 691 1973

3 Arnold H Blume K G Lohr G W Schroter W Koch H H & Wonneberger B Glucosephosphate isomerase deficiency with congenital nonspherocytic hemolytic anemia a new variant (type Nordhorn) *Pediatr Res* 8 6 1974

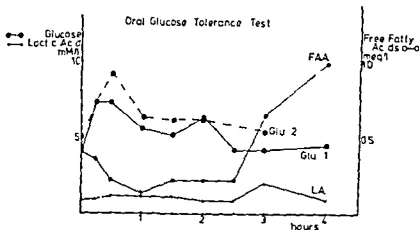


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DISCUSSION

The patient described is suffering from a generalized GPI deficiency. The enzyme deficiency was found in erythrocytes, thrombocytes, leukocytes, hepatocytes and muscle tissues. Arnold et al. (1) and Payne et al. (19) reported that no tissue specific iso-enzymes of GPI exist in man. Our observations are in

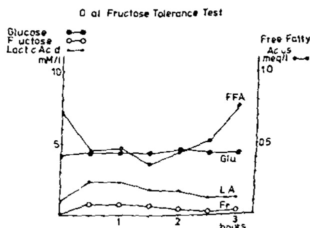


Fig. 2. The oral fructose tolerance test (- g fructose per kg bodyweight). Normal values for fructose, glucose, lactic acid and free fatty acids are seen.

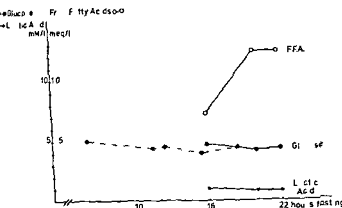


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REFERENCES

1 Arnold H Blume K G Busch D Lenkeit U Lohr G W & Lubs E Klinische und biochemische Untersuchungen zur Glucosephosphatisomerase normaler menschlicher Erythrocyten und bei Glucosephosphatisomerasemangel *Klin W chenschr* 21 1 99 1970

2 Arnold J Blume K G Engelhardt R & Lohr G W Glucosephosphate isomerase deficiency evi dence for in vi o instab lity of an enzyme variant with hemolysis *Blo d* 41 691 1973

3 Arnold H Blume K G Lohr G W Schroter W Koch H H & Wonneberger B Glucosephosphate isomerase defi iency with congenital non pherocytic hemolytic anem a a new variant (type Nordhorn) *Ped atr Res* 8 6 1974

4 Baughan M A Valentine W N Paglia D E Ways P O Simons E R & De March Q B Hereditary hemolytic anemia associated with glucose phosphate isomerase deficiency *Blood 32* 236 1968

5 Beutler E Duron O & Kelley B M *Improved* method for the determination of blood glutathione *J Lab Clin Med 61* 882 1963

6 Blume K G Hryniuk W Powars D *Trinidad F West C* & *Beutler E Characterization of two variants of glucosephosphate isomerase deficiency with* hereditary nonspherocytic anemia *J Lab Clin Med 79* 942 1972

7 Cartier P Temkine H & Griscelli C Etude biochimique d une anemie hémolytique avec deficit familial en phosphohexo isomerase *In* J Frei & M Jemelin (eds) *7th International Congress of Clinical Chemistry Geneva/Evian 1969* Karger Basel 1970 vol 2 p 139

8 Fernandes J & van de Kamer J H *Studies on the* utilisation of hexoses in liver glycogen disease *Pediatrics 35* 470 1965

9 Fernandes J Huijing F & van de Kamer J H A screening method for liver glycogen disease *Arch Dis Child 44* 311 1969

10 Hutton J J & Chilcoate R R Glucosephosphate isomerase deficiency with hereditary *nonspherocytic* hemolytic anemia *J Pediatr 85* 494 1974

11 Krone W Schneider G Schulz D Arnold H & Blume K G Detection of *phosphohexose isomerase* deficiency in human fibroblast cultures *Human genetik 10* 224 1970

12 Lowry O H Rosebrough N J Farr A L & Randall R J Protein measurement with the Folin *phenol reagent J Biol Chem 193 265 1951*

13 Lohr G W *In* E Gerlach K Moser E Deutsch W Willmanns (eds) *Erythrocytes thrombocytes leucocytes* Thieme Stuttgart 1972 p 182

14 Minakami *S C Suzuki T Saito T* & Yoshikawa W Studies on erythrocyte glycolysis *J Biochem (Tokyo) 59* 583 1965

15 Miwa S Nakashima K Oda S Oda E Matsumoto N Ogawa H & Fukumoto Y Glucosephosphate isomerase (GPI) deficiency hereditary nonspherocytic hemolytic anemia *Acta Haematol Jpn 36* 65 1973

16 Miwa S Nakashima K Oda S Matsumoto N Ogawa H Kabayashi R Kotani M Harata A Onaya T & Yamada T Glucosephosphate isomerase (GPI) deficiency hereditary nonspherocytic hemolytic anemia *Acta Haematol Jpn 36* 70 1973

17 Niessner H & Beutler E Fluorimetric analysis of glycolytic intermediates in human red blood cells *Biochem Med 8* 123 1973

18 Paglia *D E* Holland P Baughan M A & Valentine W N Occurrence of defective hexosephosphate isomerization in human erythrocytes and leukocytes *N Engl J Med 280* 66 1969

19 Payne D M Porter D W & Gracy R W Evidence against the occurrence of tissue specific variants and isoenzymes of phosphoglucose isomerase *Arch Biochem Biophys 122 122 1972*

20 *Quaife M L Scrimshaw N S & Lowry O H* Micromethod for assay of total tocopherols in blood serum *J Biol Chem 180* 1229 1949

21 *Schroter W* Brittinger *G* Zimmerschmitt E Konig E & Schrader D Combined glucose phosphate isomerase and glucose-6-phosphate dehydrogenase deficiency of the erythrocytes a new hemolytic syndrome *Br J Haematol 20* 249 1971

22 Schroter W Koch H H Wonneberger B Kalinowsky W Arnold A Blume K G & Hutter W Glucosephosphate isomerase deficiency with congenital nonspherocytic hemolytic anemia a new variant (type Nordhorn) I clinical and genetic studies *Pediatr Res 8* 18 1974

23 Slein M W Phosphohexose isomerase from muscle *In* S P Colowick & N O Kaplan (eds) *Methods in enzymology* vol I Academic Press New York 1955 p 299

24 Sigma The ultraviolet determination of 2 3 diphosphoglyceric acid in blood at 340 mμ *Sigma Techn Bull 35 UV* 12 1971

25 Tariverdian G Arnold H Blume K G Lenkeit U & Lohr G W Zur Formalgenetik der Phosphoglucoseisomerase *Humangenetik 10* 218 *1970*

26 Valentine W N Erythrocyte disorders *In* W J Williams E Beutler A J Erslev R W Rundles (eds) *Hematology* McGraw Hill New York 1972 p *405*

27 Wyss S R Koster J F & Hulsmann W C Choice of leucocyte preparation in the diagnosis of glycogen storage disease type II (Pompe s disease) *Clin Chim Acta 35* 277 1971

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CASE REPORT

MEDIASTINAL LYMPHOSARCOMA SIMULATING PERICARDITIS

MATTS BERG ERIK WILANDER and ANDERS ERIKSSON

From the Departments of Paediatrics and Pathology University Hospital Uppsala and the Department of Paediatrics Central Hospital Boden Sweden

ABSTRACT Berg M Wilander E and Eriksson A (Departments of Paediatrics and Pathology University Hospital Uppsala and Department of Paediatrics Central Hospital Boden Sweden) Mediastinal lymphosarcoma simulating pericarditis Acta Paediatr Scand 64 873 1975.—A case of lymphosarcoma of the heart in a 10-year-old boy is presented The clinical picture simulated a pericarditis. Prednisolone had a remarkably good temporary effect Post mortem examination showed a highly differentiated lymphosarcoma invading the pericardium and myocardium from a primary mediastinal localisation Cytologic examination for malignant cells in the pericardial fluid must not be forgotten in a case of unexplained pericardial effusion

KEY WORDS Lymphosarcoma heart tumour pericardial effusion

Few cardiac tumours occurring in childhood have been described in the literature Primary malignant tumours of the heart are rarities An incidence of approximately 0 08 % of all cases of congenital heart disease are seen in paediatric hospitals according to Simcha et al (4) Secondary tumours of the heart occur with at least 16 times the frequency of primary growths (2) They usually consist of lymphoma or leukaemia and commonly involve the pericardium The initial symptom of such a disease does not usually come from the heart but the clinical picture may later suggest accumulation of pericardial effu ion with a massive increase of the cardiac shadow on the chest roentgenogram The fatal outcome is often caused by congestive heart failure (7)

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The patient was a d zygotic twin boy Both twins were *mentally retarded The et ol gy of the mental retardation* is unknown H s brother is physically healthy The first cardiac symptoms were noted at the age of 9 years 9 *months The boy showed cyanosis of the lips and signs of* increased jugular venous pressure Diminished pulsation and a grossly enlarged cardiac shadow were seen at fluoroscopy The ECG showed depression of the ST segment and T wave inversion in the left precordial leads but not a particularly low voltage An exudative pericarditis was suspected The patient was treated with digitalis and diuretics and his condition improved

During the next few months the cardiac symptoms r appeared The ECG showed the same picture with additional ventricular ectopic beats Propranolol chloride and prednisolone were given The dosage was gradually tapered and finally discontinued after 2 months At that time the patient appeared to have completely recovered Chest roentgenograms showed normalisation of the heart shadow and normal cardiac pulsations were seen at fluoroscopy Digitalis therapy was continued

During the following 3 months the boy again gradually deteriorated Dyspnoea orthopnoea moderate cyanosis and hepatomegaly were noted Tran ient swelling of the neck and face was observed Roentgenological examination revealed an exudate in the right pleura The heart shadow was considerably enlarged with only minimal pulsation at fluoroscopy From the right pleura 945 cm³ of straw-coloured fluid was tapped The fluid contained 119 0 cells per 3 2 mm³ almost exclusively mononuclear cells The protein content was 4 0 g/100 ml No cytologic examination was performed At pericardial paracentesis 50 cm of serosanguineous fluid was obtained Bacterial

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6 Blume K G Hryniuk W Powars D Trinidad F West C & Beutler E Characterization of two variants of glucosephosphate isomerase deficiency with hereditary nonspherocytic anemia *J Lab Clin Med 79* 942 1972
7 Cartier P Temkine H & Griscelli C Etude biochimique d une anémie hémolytique avec déficit familial en phosphohexo-isomerase *In* J Frei & M Jemelin (eds) *7th International Congress of Clinical Chemistry Geneva/Evian 1969* Karger Basel 1970 vol 2 p 139
8 Fernandes J & van de Kamer J H *Studies on the utilisation of hexoses in liver glycogen disease Pediatrics 35* 470 1965
9 Fernandes J Huijing F & van de Kamer J H A *screening method for liver glycogen disease Arch Dis Child 44* 311 1969
10 Hutton J J & Chilcoate R R Glucosephosphate isomerase deficiency with hereditary *nonspherocytic* hemolytic anemia *J Pediatr 85* 494 1974
11 Krone W Schneider G Schulz D Arnold H & Blume K G Detection of phosphohexose isomerase *deficiency in human fibroblast cultures Humangenetik 10* 224 1970
12 Lowry O H Rosebrough N J Farr A L & Randall R J Protein measurement with the Folin *phenol reagent J Biol Chem 193* 265 1951
13 Lohr G W *In* E Gerlach K Moser E Deutsch W *Willmanns (eds) Erythrocytes thrombocytes leucocytes* Thieme Stuttgart *1972* p *182*
14 Minakami S C Suzuki T Saito T & Yoshikawa W Studies on erythrocyte glycolysis *J Biochem (Tokyo) 59* 583 1965
15 Miwa S Nakashima K Oda S Oda E Matsumoto N Ogawa H & Fukumoto Y Glucosephosphate isomerase (GPI) deficiency hereditary nonspherocytic hemolytic anemia *Acta Haematol Jpn 36* 65 1973
16 Miwa S Nakashima K Oda S Matsumoto N Ogawa H Kabayashi R Kotani M Harata A Onaya T & Yamada T Glucosephosphate isomerase (GPI) deficiency hereditary nonspherocytic hemolytic anemia *Acta Haematol Jpn 36* 70 1973
17 Niessner H & Beutler E Fluorimetric analysis of glycolytic intermediates in human red blood cells *Biochem Med 8* 123 1973
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19 Payne D M Porter D W & Gracy R W Evidence against the occurrence of tissue specific variants and isoenzymes of phosphoglucose isomerase *Arch Biochem Biophys* 122 172 1972
20 Quaise M L Scrimshaw N S & Lowry O H Micromethod for assay of total tocopherols in blood serum *J Biol Chem 180* 1229 1949
21 Schroter W Brittinger G Zimmerschmitt E Konig E & Schrader D Combined glucose phosphate isomerase and glucose-6-phosphate dehydrogenase deficiency of the erythrocytes a new hemolytic syndrome *Br J Haematol 20 249 1971*
22 Schroter W Koch H H Wonneberger B Kalinowsky W Arnold A Blume K G & Hutter W Glucosephosphate isomerase deficiency with congenital nonspherocytic hemolytic anemia a new variant (type Nordhorn) I clinical and genetic studies *Pediatr Res 8* 18 1974
23 Slein M W Phosphohexose isomerase from muscle *In* S P Colowick & N O Kaplan (eds) *Methods in enzymology* vol I Academic Press New York *1955 p* 299
24 Sigma The ultraviolet determination of 2 3 diphosphoglycenc acid in blood at 340 $m\mu$ *Sigma Techn Bull 35 UV* 12 1971
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Fig 2 Invasion of atypical lymphocytes into the pericardium (*left*) and the myocardium (*right*) van Gieson ×150

superior vena cava and right atrium is highly suggestive of a tumour obstructing the venous inflow Cytologic examination for malignant cells in the pericardial fluid is undoubtedly the most direct method by which the diagnosis of pericardial malignant lesion can be established (6) Unfortunately this examination was not performed in the present case

In two different groups of children with lymphosarcoma 104 cases in all reported by Dargeon (1) and Sullivan (5) none had initial cardiac symptoms The most frequent locations of initial symptoms were the abdomen and the retroperitoneal region with pain and tumefaction as the most common manifestation Our patient had no visible or palpable tumefaction of the peripheral lymph nodes The mediastinal glands did not seem to be enlarged on the chest roentgenogram and the discovery of invasively growing lymphosarcoma was a surprise If the exact diagnosis had been established by other means the therapeutic approach would have been different

Six months before he died our patient s symptoms had regressed considerably during a two month course of treatment with prednisolone and propranolol chloride A similar remarkably good temporary effect of prednisone therapy has been reported by Pader (3) in a case of pericardial effusion due to primary sarcoma of the pericardium

The tumour tissue which was a highly differentiated lymphosarcoma seemed to have invaded the pericardium and myocardium by direct extension from a primary mediastinal localisation The result was an impairment of cardiac function partly by a constricting effect



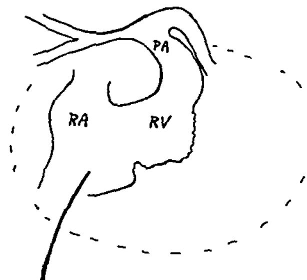


Fig 1 Right atrial postero anterior angiogram

cultures including tests for tuberculosis were all negative Laboratory investigations gave the following values Hb 12 8 g/100 ml hematocrit 45 vol% and WBC 10000/mm³ (5600 polynuclear and 4400 mononuclear cells) ESR was 23 mm/hr Urinanalysis gave normal results Lupus erythematosus cell preparation antinuclear factor antigamma globulin and acryl tests were negative Liver enzymes serum electrolytes and serum creatinine were within normal limits Serum protein was 8 2 g/100 ml Serum electrophoresis showed slight unspecific signs of activity with increased immunoglobulins

At cardiac catheterization the mean pressure in the right atrium was 13 and in the superior vena cava 22 mmHg The right ventricular pressure was 40/17 mmHg thus there was an increase in the end-diastolic value Angiography with injection of contrast medium into the right atrium revealed signs of a considerable quantity of fluid or other content in the pericardium The inner contour of both atria was situated approximately 2 cm from the outer contour of the heart shadow The right ventricle was displaced somewhat to the left A constriction of the proximal 3 cm of the right pulmonary artery was seen (Fig 1)

The patient s condition now deteriorated rapidly The only therapeutic alternative was considered to be explorative thoracotomy At the beginning of the anaesthesia after intubation severe bradycardia developed and the blood pressure fell During the resuscitation with the chest opened a mediastinal tumour with overgrowth to the pericardium was observed The heart could not be brought to spontaneous activity again When the patient died 10 months had passed from the time when the first symptoms of the disease were manifested

Post mortem examination showed a widened mediastinum filled with a greyish white glossy tumour tissue which extended from the aperture of the upper thorax through the mediastinum to the heart The right and left pulmonary arteries and the superior vena cava were all constricted and had only pencil wide luminae

The tumour tissue also extended to the parietal and visceral pericardium and formed membranes up to 1 cm thick which were covered with blood and fibrin The tumour did not invade the lung tissue or pleurae The myocardium was loose and flabby Microscopically the tissue of the lymphatic glands in the mediastinum had a normal but destroyed architecture and was completely invaded by a homogeneous infiltrate of small irregular hyperchromatic lymphocytes which penetrated the capsules of the lymphatic glands and merged with the tumour tissue in the surroundings which consisted of atypical lymphocytes of the same appearance as those in the lymphatic glands The myocardial tissue showed degenerative changes with splitting and diffuse invasion by atypical lymphocytes which merged into a homogeneous tissue subpericardially (Fig 2)

DISCUSSION

In this patient the heart symptoms predominated from the beginning The clinical picture with venous stasis pleural effusion an enlarged heart shadow with diminished pulsation and angiographic signs of a considerable quantity of fluid in the pericardium simulated a pericarditis

Cardiac catheterization is a valuable procedure in investigating patients in whom constrictive pericarditis is suspected In a patient with unexplained pericardial effusion a tumorous origin should be considered The finding of a pressure gradient between the

CASE REPORT

ACUTE LIVER FAILURE AND ENCEPHALOPATHY (REYE S SYNDROME?) DURING SALICYLATE THERAPY

MATTI SILLANPAA ANNA LIISA MAKELA and ANTTI KOIVIKKO

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ABSTRACT Sillanpaa M Makela A L and Koivikko A (Department of Paediatrics University of Turku Turku Finland) Acute liver failure and encephalopathy (Reye s syndrome) *during salicylate therapy* Acta Paediatr Scand 64 877 1975.—A case of hepatotoxicity and encephalopathy (Reye s syndrome) associated with salicylate therapy is presented and the aetiology of this syndrome is discussed Hepatotoxicity developed with salicylate serum concentrations not exceeding therapeutic serum levels The importance of controlling serum salicylate concentration and transaminase activity particularly during the first fourteen days of therapy is emphasized

KEY WORDS Reye s syndrome salicylate therapy drug induced hepatotoxicity

:ylate induced hepatotoxicity is well *mented in the literature (2 5 8 10 11* Clinical features with acute liver failure encephalopathy can resemble those of e s syndrome

ı the following report a case with this sud and unexpected complication during sali ıte therapy is presented

CASE REPORT

3 year-old girl presented with a 2 month history of teral finger and ankle joint pain In the initial period e was fever of 39°C which receded with small doses cetosalicylic acid and her joints became symptomless week before admission to hospital she again became nle with accompanying joint pains in her ankles and es and later on in her fingers and wrist joints On mission to hospital her general condition was good but had mild fever and remarkable joint s gns hot and der wrists and oedematous toes and fingers with defec e clenching and in addition hydrops of one knee eurological status was normal

Investigations erythrocyte sedimentation rate (ESR) 3 mm/h Hb 116 g/l C-reactive protein strongly posi e antinuclear factors negati e serum lactic acid dehydrogenase (S LD) 560 U/l (normal 260–600 U/l) serum glutamic oxalacetic transaminase (S ASAT SGOT) 14 U/l (normal below 35 U/l) plasma thromboblastin time (P T plast) 0 95 (normal 0 70–1 30) Waaler Rose test below 1 32 antistreptolysin titre (AST) 90 antistaphylolysin titre (ASTA) 3 0–2 5 Serum iron 5 µmol/l iron binding capacity 49 5 µmol/l IgG 21 g/l IgA 3 9 g/l and IgM 1 8 g/l Serum bilirubin 9 µmol/l

The patient received microcrystalline acetyl sali ylic acid (Medisyl® Medica) 82 mg/kg body weight or 4 g daily divided into three doses Serum salicylate concentration (S Salis) was measured daily (Fig 1)

The joint signs disappeared in a few days during the salicylate therapy but the patient became subfebrile On the eighth day of the treatment she developed rhinorrhea and on the tenth day a *pyrexia* of 38 7°C On the following day she suddenly became confused and the salicylate therapy was withdrawn The next morning she was restless and delirious for a short period with deep and rapid respiration She vomited blood and complained of vertigo and shortly afterwards lost consciousness and developed convulsions There was spasticity in all limbs and both plantar responses were extensor Painful stimuli elicited elementary reflex responses The pupils were dilated and reacted sluggishly Fundoscopy was normal

Laboratory examinations showed the following pathological findings S-LD was 10500 U/l S-ASAT 250(U/l serum ammonia (fp-Ammon) 130 µmol/l (normal below 50 µmol/l) P T plast 0 .6 and serum total bilirubin 2 µmol/litre The values of S-ASAT and S-LD are shown ı Fig 1 Cerebrospinal fluid was normal

of the thick membranes of the pericardium and partly by invasion of the tumour into the actual heart tissue, affecting the muscle sufficiently to produce congestive failure The encroachment upon the superior vena cava explained the previous symptoms of Stokes's collar

REFERENCES

1 Dargeon H W Lymphosarcoma in childhood *Am J Roentgenol 85* 729 1961
2 Griffith G C A review of primary tumors of the heart *Progr Cardiovasc Dis 7* 465 1965
3 Pader E & Kirschner P A Primary sarcoma of the pericardium *Am J Cardiol 14* 399 1964
4 Simcha A Wells B G Tynan M J & Waterston D J Primary cardiac tumours in childhood *Arch Dis Child 46* 508 1971
5 Sullivan M P Leukemic transformation in lymphosarcoma of childhood *Pediatrics 29* 589 1962
6 Thurber D L Edwards J E & Achor R W Secondary malignant tumors of the pericardium *Circulation 26* 228 1962
7 Van der Hauwaert L G Cardiac tumours in childhood *In* Watson H (ed) *Paediatric Cardiology* Lloyd Luke London 1968 p 773

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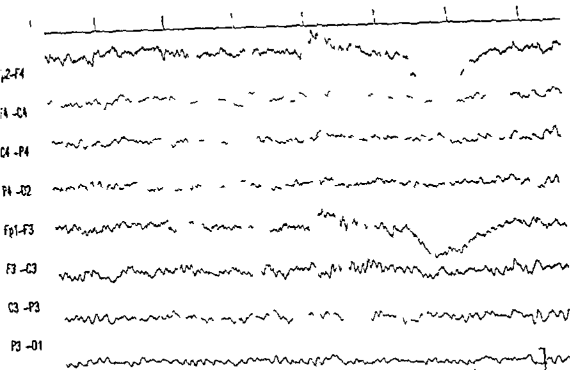


Fig 3 EEG a few hours after the arousal of the patient Regular occipital rhythmic alpha activity (8 Hz 50 μV) no definite reaction to closing or opening of the eyes Diffuse low amplitude slow background activity and base line instability with some sharp transients Calibr 1 sec 100 μV

During the course of the illness blood glucose serum creatinine sodium and potassium acid–base balance and bone marrow smear were all normal

No viruses were isolated No antigens could be detected for hepatitis B Ebstein Barr Adeno Influenza A and B Cytomegalo Echo 6 Coxsackie A 9 Coxsackie B 5 Mycoplasma pneumoniae Yersinia or Salmonella infections Australia antigen was also negative

After 10 months the patient is still symptomless and is not receiving any medication There has been no recurrence of joint symptoms

DISCUSSION

The case we report demonstrated the typical features of Reye s syndrome but without the acidosis and hypoglycaemia Liver biopsy was not performed and the diagnosis remains uncertain

The aetiology of Reye s syndrome has remained controversial ever since the syndrome was first described in the literature (9) While many authors mention prodromal upper respiratory tract infection (1 2 3 4 6 7 9) increasing attention has been attached to the possible role of salicylates as an aetiological factor either alone (8) or together with coexistent respiratory tract infection (12) Nasal congestion and slight hyperpyrexia may also be intial symptoms of salicylism which further confuses the situation Our patient also had these prodromal symptoms before the onset of coma

A notable finding in our case was the abnormal elevation of serum glutamic oxalacetic transaminase activity found on the tenth day following initiation of salicylate therapy Iancu & Elian (5) have previously drawn attention to the fact that serum glutamic oxalacetic and glutamic pyruvic transaminase elevations occurring after 10 days of salicylate therapy are an early sign of tissue damage and emphasized the importance of regular determination of these enzymes together with that of blood salicylate levels to prevent further hepatic damage and also possible bleeding

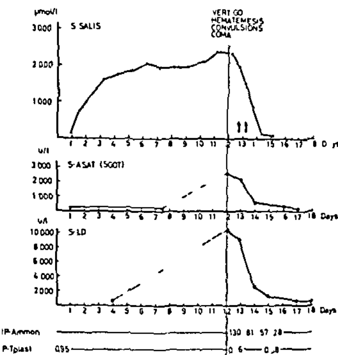


Fig 1 Serum salicylate concentration (S salis) S LD S ASAT fP Ammon and P T plast during the critical days of salicylate treatment

An EEG performed when the patient was comatose exhibited strong general slowing and low amplitude activity intermingled with occasional spindle activity (Fig 2)

During the comatose state two exchange transfusions were performed with altogether 5000 ml of blood The *total duration of coma was 60 hours The patient then* suddenly regained consciousness and was well orientated and responsive to verbal communication The improvement was rapid and in few days she became completely symptomless Liver enlargement which was found during the coma disappeared as well as bleedings into mucous membranes the latter after intramuscular injection of vitamin K The rest of her treatment included peroral Lipotion® (1 2 ditiolan 3 valerylamide 4 uracilcarboxyl acid dehydrochol acid vitamin B complex vitamin C and inositole) intravenous dexamethasone 8 mg three times a day and intravenous diazepam for convulsions In the course of treatment the abnormally high enzyme activities returned to normal (Fig 1)

A second EEG was performed coexistently with the arousal of the patient and showed almost regular occipital rhythmic activity of alpha variety but still with abnormally increased slow background activity (Fig 3) A fortnight later there were intermittent slow spike and wave discharges of 1–2 sec duration in an otherwise normal EEG Two months after the coma the EEG was again normal

Urine amino acid analysis showed elevated leucine isoleucine fraction 9 days after the onset of the comatose state

Fp2-F4
F4 -C4
C4 -P4
P4 -O2
Fp1-F3
F3 -C3
C3 -P3
P3 -O1

Fig 2 EEG during the comatose state (17th day) Sub delta activity with low amplitude and intermittent bilateral spindles Calibr 1 sec 100 μV

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CASE REPORT

GENERALIZED MYOPATHY AND CEREBRAL MALFORMATIONS POSSIBLY RELATED TO AN ENTEROVIRAL INFECTION

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ABSTRACT Hansson O Kristensson K Lycke E Solymar L and Sourander P (Departments of Paediatrics II Virology and Pathology I University of Gothenburg Swe den) Generalized myopathy and cerebral malformations possibly related to an enteroviral infection Acta Paediatr Scand 64 881 1975 —A study was made on a case of generalized muscular hypotonus manifested at birth Serological findings and epidemiological data sug gested an association to a recently described enterovirus infection (enterovirus candidate 71) known to cause neurological disease in man Autopsy revealed cerebral malformations and generalized myopathy compatible with a viral etiology of the disease

KEY WORDS Congenital myopathy cerebral malformations enterovirus infection

A case of a prenatally acquired myopathy and cerebral malformations is presented In 1973 an epidemic of aseptic meningitis oc curred in Southern Sweden It was caused by a previously rarely recognized enterovirus (1) The possibility that the disease of the child was etiologically associated with this en terovirus infection is discussed

CASE REPORT AND METHODS

History and clinical findings

A boy born in June 1973 without heredity for neurologi cal or muscular disorders demonstrated at birth a re markably generalized muscular hypotonus and practically no spontaneous movements of the limbs From the sixth day of life gastric tube feeding was necessitated

At admission to the department of paediatrics at the age of one month the boy showed a pronounced muscular hypotonus of the trunk and limbs Except for some weak voluntary movements of fingers toes and proximal mus cles of the limbs no movements were observed The position of the face was in extension and the hands in flexion due to slight contractures The patellar reflex was symmetrical and normal No other deep reflexes were elicited The abdominal reflexes were absent The anal sphincter reflex was normal Superficial pain sensation was normal No cranial nerve abnormality or fascicula tions in the tongue or elsewhere were noted The respira tory muscle function was poor but there was no cyanosis The cry was weak No other abnormalities were found at repeated physical examinations The boy died at the age of 6 weeks

Neurophysiological investigations EMG showed no signs of denervation potentials but a great number of polyphasic potentials in m quadriceps and m tibialis anterior Echoencephalography was normal EEG was not performed

Biochemical investigations Creatinphosphokinase 930 U/l (normal <300) GOT 143 89 U/l (normal <60) Lactic dehydrogenase 598 U/l (<450) GPT and immunoglobulins (G A and M) were normal

Other clinical investigations X ray of the skull spine lungs and heart were normal ECG was normal

Histology For histological examination paraffin embedded sections were cut from the frontal orbital central and occipital areas of the cerebral lobes cerebel lum pons medulla oblongata spinal cord end roots sciatic and femoral nerves They were stained with van Gieson HTX-eosin luxol fast blue–cresyl violet Ranke s method for glia fibres and impregnated with silver accord ing to Palmgren Specimens from the quadriceps biceps and deltoid muscle and tongue were available for examina tion

In agreement with the findings in our case the data in the literature show that serum salicylate concentrations seldom exceed the intended therapeutic level in cases of Reye s syndrome (6 12, 13)

REFERENCES

1 Becroft D M O Syndrome of encephalopathy and fatty degeneration of viscera in New Zealand children *Br Med J II* 135 1966
2 Giles H McC Encephalopathy and fatty degeneration of the viscera *Lancet I* 1075 1965
3 Glick T H Likosky W H Levitt L P Mellin H & Reynolds D W Reye s syndrome an epidemiologic approach *Pediatrics 46* 371 1970
4 Huttenlocher P R Schwartz A D & Klatskin G Reye s syndrome ammonia intoxication as a possible factor in the encephalopathy *Pediatrics 43* 443 1969
5 Iancu T & Elian E Aspirin induced abnormalities of liver function *Am J Dis Child 128* 116 1974
6 Norman M G Encephalopathy and fatty degeneration of the viscera in childhood I Review of cases at the Hospital for Sick Children Toronto (1954–1966) *Can Med Assoc J 99* 522 1968
7 Olson L C Bourgeois C H Jr Cotton R B Hankul S Grossman R A & Smith T J Encephalopathy and fatty degeneration of the viscera i northeastern Thailand Clinical syndrome an epidemiology *Pediatrics 47* 707 1971
8 Shaw E B Reye s syndrome and salicylate intoxication *Pediatrics 46* 976 1970
9 Reye R D K Morgan G & Baral J Encephalopathy and fatty degeneration of the viscera A disease entity in childhood *Lancet II* 749 1963
10 Russell A S Sturge R A & Smith M A Serum *transaminases during salicylate therapy Br Med J II* 428 1971
11 Seaman W E Plotz P H & Ishak K G Aspirin induced hepatotoxicity in patients with RA o systemic lupus erythematosus (SLE) *Arthritis Rheum 17* 325 1974
12 Silverman A Roy C C & Cozzetto F J *Pediatric clinical gastroenterology* Mosby St Loui 1971 p 345
13 Utian H L Wagner J M & Sichel R J S White liver disease *Lancet II* 1043 1964

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Fig 1 Base of the brain showing pachygyria in temporal and occipital lobes and micro-polygyria in fronto-orbital cortex

inferior part of the frontal lobes numerous microgyria were seen In other parts of the hemispheres the gyri were somewhat smaller than normally

In coronal sections through the brain the borderline between grey and white matter was indistinct This was especially the case in the occipital lobes where the medulla had a greyish discoloration The basal ganglia brainstem and cerebellum showed no obvious changes The ventricular system choroid plexus and ependyma were normal The basal arteries showed no changes

In the occipital cortex the normal lamination was lost (Fig 2) The thickness of the cortex showed marked variation with an ill-defined border to the white matter This was for the most part devoid of myelin and contained numerous heterotopic islands of grey matter separated by fibrillary glial tissue The walls of



Fig 2 Low power view of occipital cortex showing severely disorganized cytoarchitectonic and abnormal vessels surrounded by scar tissue Cresyl violet–Luxol fast blue

the cortical vessels were somewhat thickened and surrounded by glio mesenchymal scar tissue The disorganization of the cortical cytoarchitectonic was particularly marked around such vessels (Fig 2) Similar changes of the cortex were seen in the inferior frontal lobes with the areas of microgyria The leptomeninges showed areas of fibrosis and a slight infiltration of mononuclear inflammatory cells No significant changes were seen in the brain stem and cerebellum The spinal cord was normal No changes were seen in the motor neurons spinal roots or peripheral nerves

Specimens from the quadriceps biceps and

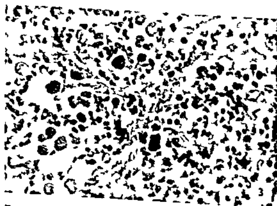


Fig 3 Quadriceps muscle showing groups of fibres with small diameter but without signs of degeneration HTX eosin

Table 1 *Serological findings in blood samples from mother and child*
CF=complement fixation test HI=hemagglutination inhibition test DT=dye test NT=neutralization test

	Serum sample from antibody titres in			
	Child	Mother		
Antigen	73 06 27	73 11 07	74 03 12	74 05 14
CMW (CF)	<4	8	4	4
HSV (CF)	<4	< 4	N D	N D
Coxsackie B5 (CF)	<4	< 4	N D	N D
Rubella (HI)	N D	<10	N D	N D
Toxoplasma (CF)	N D	< 4	N D	N D
Toxoplasma (DT)	N D	<10	N D	N D
Enterovirus F J (NT) (enterovirus 71)	80	20	10	20

Virology From the child two small fragments one of the quadriceps muscle and one of fat tissue were available for virus isolation The specimens left over from neurophysiological investigations had been kept frozen at −20° for more than 3 months before attempts to isolate virus were made Virus isolation was attempted using a number of cell cultures which were observed for one month

Blood specimens from child and mother were examined serologically The specimen from the child was obtained July 27 and those from the mother November 7 1973 March 12 and May 14 1974 i e the blood sample from the boy was taken when he was 1½ months old and the samples from the mother originated from 5 8 and 11 months respectively after the child was born The child s serum was studied in complement fixation tests (CF) against cytomegalovirus (CMV) herpes simplex virus (HSV) and Coxsackie B 5 virus antigens CF tests were also performed against these antigens on the mother s serum and in addition CF and dye tests against toxoplasma antigens and hemagglutination inhibition (HI) tests against rubella were made on the blood specimens of the mother for demonstration of toxoplasma and rubella antibodies

Sera from both mother and child were observed for neutralizing antibodies against a Swedish enterovirus strain (F J) immunologically closely related to the enterovirus strain BrCr (enterovirus candidate 71) (1)

RESULTS

Virology

No virus was isolated from the tissue fragments available It should be remembered however, that there were only minute amounts of the tissues available and that the specimens had been stored for more than 3 months before attempts to isolate virus were performed

The serological studies (Table 1) failed to demonstrate antibodies in mother and/or child against HSV Coxsackie B5 rubella or toxoplasma antigens The findings of CMV antibodies did not emphasize a possible association between CMV infection and the disease discussed Thus no antibodies against CMV were observed in the child and the titres of the mother s sera were persistently low

The small amounts of the serum available from the child did not permit a survey of antibody prevalence There were however as mentioned no antibodies against CMV HSV or Coxsackie B 5 antigens detectable On the other hand both mother and child demonstrated neutralizing and the mother in addition CF antibodies against enterovirus F J The difference in neutralizing antibody titres indicated a four fold higher titre in the child than in the mother This observation is suggestive of an immunization of the child and is not directly compatible with the assumption merely of transfer of maternal antibodies

Pathology

General autopsy showed tracheobronchitis and pulmonary oedema but no other significant changes The brain weighed 700 g It showed a marked abnormality in the gyral pattern with relatively symmetrical changes in the basal parts of the cerebral hemispheres The medial and basal aspects of the occipital and temporal lobes displayed pachygyria with few broad gyri and shallow sulci Over a large area the gyri were absent altogether (Fig 1) In the

sation in association with such altered vessels It is therefore possible that the cortical changes with gyral abnormalities are secondary to such vascular changes The histological picture was not compatible with micropolygyria Schob (5) suggested the eponym pseudopachygyria for gyral abnormalities resembling pachygyria but due to healed destructive processes

In conclusion the possibility exists that the unusual neonatal disease in the present case affecting both the brain and muscles was caused by an in Sweden uncommon enterovirus infection which during the epidemic was known to cause cases of meningitis and meningo-encephalitis

REFERENCES

1 Blomberg J Lycke E Ahlfors K Johnsson T Wolontis S & v Zeipel G New enterovirus type associated with epidemic of aseptic meningitis and/or hand foot and mouth disease *Lancet II* 11 1974

2 Kemper T L Lecours A R Gates M J & Yakovlev P I Retardation of the myelo- and cytoarchitectonic maturation of the brain in the congenital rubella syndrome *Res Publ Assoc Res Nerv Ment Dis 51* 23 1973

3 Rorke L B & Spiro A J Cerebral lesions in congenital rubella syndrome *J Pediatrics 70* 243 1967

4 Schmidt N J Lenette E H & Ho H H An apparently new enterovirus isolated from patients with disease of the central nervous system *J Infect Dis 129* 304–309 1974

5 Schob F Pathologische Anatomie der Idiotie *In* O Bumke (ed) *Handb Geisteskr Spe Teil VII* Springer Berlin 1930 p 837

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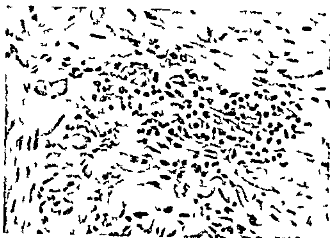


Fig 4 Slight infiltration with lymphocytes and histiocytes between apparently well preserved muscle fibres of the tongue HTX-eosin

deltoid muscle and the tongue were available for examination (Figs 3, 4)

Most muscle fibres had a normal diameter Fibres of small diameter, possible atrophic and arranged in groups were found In such areas there was an increase in the endomysial connective tissue Hypertrophic fibres were not seen The muscle fibres showed no degenerative changes Occasionally large vesicular nuclei with prominent nucleoli were seen but there were otherwise no signs of regeneration No fat infiltration was seen

Around a few blood vessels mainly in the perimysial tissue a slight infiltration of lymphocytes histiocytes and a few neutrophilic leukocytes were seen The infiltrations were perivascular and the vessels appeared to be intact

DISCUSSION

The clinical picture of the present case was dominated by generalized muscular hypotonus Biochemical neurophysiological and histological data indicated a primary myopathy

Infantile spinal muscular atrophy (Werdnig Hoffmann disease) could be ruled out since there were no changes of the motor nerve cell bodies or the ventral roots No changes were seen in large peripheral nerves as revealed by the light microscopical examination There was no evidence of muscular dystrophy or of any other hereditary myopathy

The most evident changes were a moderate peri and endomysial fibrosis and a slight perivascular infiltration of inflammatory cells The lack of muscle fibre degeneration makes it impossible to establish a firm diagnosis of polymyositis The picture might however be consistent with a low grade interstitial myositis which may be seen in connection with a large group of various diseases

The serological findings indicated that both child and mother were infected by a new enterovirus prevalent at the time of gestation (1) Strains isolated from the 1973 Swedish epidemic were found to correspond immunologically to the BrCr strain (enterovirus candidate 71) reported by Schmidt et al (4) There is much evidence that this virus produces CNS disease in man (4) and during the Swedish epidemic the relative number of small children affected with CNS infection was alarming The time difference for the sampling of sera from child and mother might invalidate a statement about the etiological relation between the enterovirus infection and the disease of the child It is possible although not probable that the lower antibody titre in the mother reflects a decrease in antibody level occurring during the period of time between the samplings from child and mother

The pathogenesis of virus induced changes in the developing brain is still incompletely known In rubella virus infections the changes include low brain weight and delayed maturation of the cortical cytoarchitectonic and myelination These changes might reflect a direct effect of persistent virus infection of the neuroectodermal cells during foetal and early postnatal development (2) However brain lesions might also be secondary to observed damage of the cerebral vessels (3) In the present case marked changes of the leptomeningeal and intracerebral vessels were observed The cortex showed gliotic scars and disorgani

sation in association with such altered vessels It is therefore possible that the cortical changes with gyral abnormalities are secondary to such vascular changes The histological picture was not compatible with micropolygyria Schob (5) suggested the eponym pseudopachygyria for gyral abnormalities resembling pachygyria but due to healed destructive processes

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2 Kemper T L Lecours A R Gates M J & Yakovlev P I Retardation of the myelo- and cytoarchitectonic maturation of the brain in the congenital rubella syndrome *Res Publ Assoc Res Nerv Ment Dis 51* 23 1973

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Fig 4 Slight infiltration with lymphocytes and histiocytes between apparently well preserved muscle fibres of the tongue HTX eosin

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3 Rorke L B & Spiro A J Cerebral lesions in congenital rubella syndrome *J Pediatrics 70* 43 1967

4 Schmidt N J Lenette E H & Ho H H An apparently new enterovirus isolated from patients with disease of the central nervous system *J Infect Dis 129* 304–309 1974

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CASE REPORT

VARICELLA FOLLOWED BY GLOMERULONEPHRITIS

Treatment with Corticosteroids and Azathioprine Resulting in Recurrence of Varicella

F KARUP PEDERSEN and E ANTONI PETERSEN

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ABSTRACT Pedersen F K and Petersen, E A (University Clinic of Infectious Diseases Blegdamshospitalet Copenhagen Denmark) Varicella followed by glomerulonephritis. Treatment with corticosteroids and azathioprine resulting in recurrence of varicella Acta *Paediatr Scand 64 886 1975* —The present report outlines the clinical features of a 2 year-old boy who following varicella developed purpura of the lower extremities transient gastrointestinal bleeding and glomerulonephritis The triad of symptoms suggests Schonlein Henoch Syndrome but coagulation studies and renal biopsy did not confirm this and varicella is thought to be the cause of the complications Therapy with corticosteroids and *azathioprine* had only a minor effect on the nephritis but caused depression of serum IgG and specific antibody resulting in reinfection or reactivation of varicella

KEY WORDS Varicella complications, varicella reactivation glomerulonephritis purpura fulminans gastrointestinal bleeding

Varicella in childhood is usually a self-limiting disease with relatively few and benign complications (1) but serious complications do occur Bleeding tendency due to thrombocytopenia (10) as well as hemorrhagic varicella elements caused by decreased capillary resistance (12) and purpura fulminans with disseminated intravascular coagulation (7) have been described Also isolated gastrointestinal bleeding has been seen (3) Nephritis due to varicella has been suspected now and then since Henoch s first publication on the subject 1884 (6) but only in recent years have reports with documentation of etiologic relationship and description of renal histology appeared (9, 11 16) Treatment with cyclophosphamide and corticosteroids has recently been reported to cause reactivation of varicella (13) and recurrence has also been assumed following steroid therapy alone (2)

CASE REPORT

Patient R N a 2 year-old boy previously in good health was admitted in November 1973 because of hemorrhagic exanthema of the lower extremities occurring within the latest 2 days Sixteen days before admission the patient had onset of low grade fever and generalized vesicular eruption and was seen by the family physician who diagnosed typical varicella that lasted up to the admission when crusts were still present Nine days before admission there was an episode of earache supposedly caused by a suppurative otitis and treated with penicillin for 6 days

On admission the patient was afebrile and not in acute distress with diffusely scattered varicella crusts and in the lower extremities additional petecchial elements and

Fig 1 Silver Methenamine+Hematoxylin Eosin 2 μm section original magnification ×416 Epithelial crescent in Bowman s capsule The glomerular tuft is partly obliterated

Fig 2 Silver Methenamine+Hematoxylin Eosin ‐ μm section original magnification ×416 Slightly hypercellular glomerulus with some double contoured basement membranes


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2

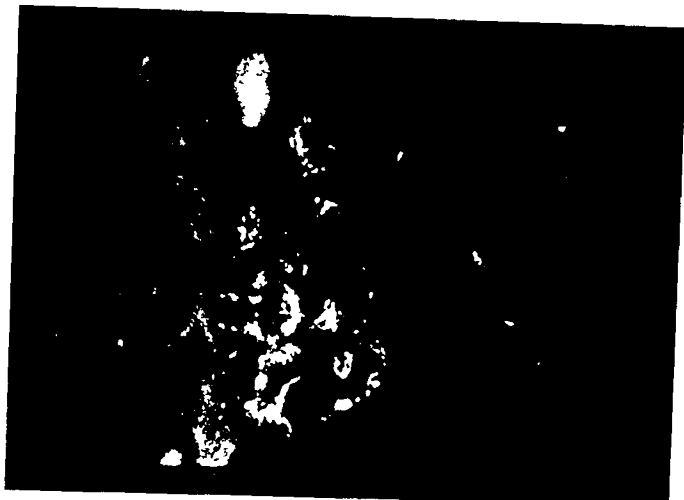


Fig 3 Glomerulus showing granular deposits of IgA in the mesangium at fluorescent microscopy

ecchymoses the latter especially localised to ankles and feet There was moderate edema of hands and feet but no arthritis and no evidence of focal infection including otitis Body weight was 13 kg Initial laboratory studies showed hemoglobin 10 5 g/100 ml leucocytes 18 9 10^9/l differential count normal thrombocytes 511 10^9/l prothrombin 0 65% of the mean normal value bleeding time and coagulation time normal fibrinogen determination failed in laboratory fibrinogen related antigens above 160 mg/l (normally below 2 5 mg/l) and increased fibrinogen monomers Urine analysis electrolytes creatinine and blood pressure were normal Cultures from throat blood and urine were negative for pathogenic micro organisms

No new hemorrhagic skin elements were observed in the hospital and varicella crusts as well as hemorrhagic lesions disappeared within a week

On admission stools were negative for blood but after 2 days the patient showed evidence of abdominal pain and passed several bloody benzidine positive stools There was a concomitant drop in Hb values to 8 9 g/100 ml and stools remained positive for blood for 7 days but thereafter were persistently negative Cultures for enteric pathogenic bacteria were negative Intestinal X ray series were not performed

Two days after admission onset of proteinuria was observed and after a few more days erythrocytes and hyaline casts appeared in the urine The proteinuria was of the order 2–4 g/24 h there was 10–50 erythrocytes per field creatinine and blood pressure were and remained normal serum albumen was 3 3 g/100 ml and edema was not observed Serum levels of complement C3 and C4 were not decreased and titres of antistreptolysin O and antistreptococcal hyaluronidase as well as antistaphylolysin were and remained normal

After 6 weeks a renal biopsy was performed The specimen for light microscopy contained 40 corpuscles More than half of the Bowman s capsules showed epithelial proliferation with formation of crescents and in several places there was adherence between capsule and tuft (Fig 1) The glomeruli showed diffuse hypercellularity with hyperplasia of mesangial cells A few glomeruli were normal There was no infiltration of granulocytes but an increased number of mononuclear cells were found in the capillaries The basement membranes were slightly irregular and showed occasional double contours (Fig 2) There was no evidence of interstitial nephritis and vessels and tubules appeared essentially normal The histological diagnosis was moderate to severe extracapillary glomerulonephritis Immunofluorescent studies showed IgA IgG and IgM localised in a granular manner in the mesangium (Fig 3) but practically none along the basement membrane and no IgA in the arterioles C3 was seen to follow the immunoglobulins Fibrinogen was found in the mesangium as well as along the basement membrane

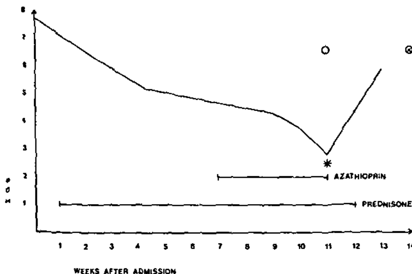


Fig 4 Serum IgG and varicella complement fixation titer in relation to treatment with prednisone and azathioprine

At the onset of nephritis therapy with prednisone 5 mg twice daily was begun but as no significant reduction in proteinuria occurred azathioprine 25 mg daily was added after 7 weeks This was followed by a decrea e in serum IgG to 2 8 g/l and specific varicella antibodies measured by complement fixation titre were found less than 1 8 (Fig 4) Lymphocyte counts remained relatively unaffected never falling below 3 0 10⁹/l After 4 weeks of combined therapy typical varicella lesions reappeared on the back chest abdomen and extremities of the patient accompanied by a rise in temperature Electron microscopy of vesicle content visualised herpes type virus and cultures from vesicle fluid yielded varicella viru There was a significant rise in varicella complement fixation titre to 1 1 8 whereas herpes simplex titre did not show any rise Azathioprine was discontinued and prednisone tapered off over a period of 1 week and the patient underwent an uneventful recovery from the varicella Immunoglobulin level returned to normal within ? weeks and lymphocyte transformation studies done 3 weeks after discontinuation of the drugs showed normal response to stimulation with phytohemagglutinin pokeweed mitogen and specific microbial antigens from *Cana da albicans E c li Staph lo occus aureus* Streptococcus and PPD During therapy proteinuria was reduced to the order of 1 0–1 5 g/24 h while erythrocyturia was not affected and urinary abnormalities have remained essentially unchanged since then e for 5 months without treatment

DISCUSSION

The triad of symptoms in the present case *may* suggest Schonlein Henoch Syndrome (SHS) The laboratory evidence of recent intravascular coagulation however is more in favour of the purpura being a mild form of purpura fulminans than SHS Glomerular changes in SHS nephritis are reported as being focal within both the single glomerulus and in distribution with many normal glomeruli present (15) whereas histological changes in varicella nephritis are diffuse (16 11 9) as was the case in the present patient The localisation of immunoglobulins primarily in the mesangium *rather than in* the basement membrane is seen in SHS nephritis but usually associated with IgA deposits in the small arterioles (14) These were not present in our patient and previous reports of immunofluorescent studies of varicella nephritis are not available Thus it appears unlikely that the observed complications represent SHS In any case it may be speculated that SHS as well as the varicella complications could represent different manifestations of immune complex disease with varicella virus as antigen and that the differentiation may therefore be of minor importance

The etiologic relationship of the complications to varicella of course remains presumptive but the preceding attack of varicella was *typical* of the disease and though no comple

ment fixation titre was obtained during the initial episode, the fact that the patient stayed in the varicella ward constantly exposed for 2½ months without contracting the disease before immunoglobulins became depressed is strong evidence that the initial disease was actually varicella In spite of a history of otitis no evidence of preceding streptococcal infection could be shown by repeated serological studies and complement levels were normal, contrary to what is seen in poststreptococcal nephritis

In view of the continued urinary abnormalities and the severe changes in the renal biopsy in our patient combined therapy with corticosteroids and azathioprine was attempted As seen in Fig 4, serum IgG became depressed and varicella complement fixation titre was found below 1 8 after 4 weeks of combined therapy, whereas lymphocyte counts remained relatively unaffected Reinfection or reactivation of varicella followed suggesting that antibodies play a greater role in protection against the disease than lymphocyte function Although several reports point to the severe and potentially fatal course of varicella in patients during treatment with steroids (4 5) and immunosuppressive agents (8) our patient recovered within about a week after discontinuation of therapy

The present report adds to already existing evidence that varicella can be the cause of glomerulonephritis and it documents reinfection or reactivation of varicella during treatment with corticosteroids and azathioprine In view of the lack of definite proof that cytostatic agents are of value in treatment of glomerulonephritis and considering reports of severe course and fatalities in varicella patients receiving such drugs we suggest that they be withheld in patients with varicella nephritis, in whom risk of reactivation of varicella does exist

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REFERENCES

1 Bullowa J G M & Wishik M Complications of varicella I Their occurrence among 2534 patients *Am J Dis Child 49* 923 1935
2 Falliers C J & Ellis E F Corticosteroids and varicella *Arch Dis Child 40* 593 1965
3 Farhoudi H O Nash E C & Desai R GI bleeding as a complication of varicella *Postgrad Med 54* 181 1973
4 Gershon A Brunell P A & Doyle E F Steroid therapy and varicella *J Pediatr 81* 1034 1972
5 Haggerty R J & Eley R C Varicella and cortisone *Pediatrics 18* 160 1956
6 Henoch E Nephritis nach Varicellen *Berliner Klin Wchnschr 21* 17 1884
7 Horder M Purpura fulminans following varicella *Dan Med Bull 15* 221 1968
8 Kobayashi A & Ohbe Y Nephrosis cyclophosphamide therapy and varicella *J Pediatr 81* 1034 1972
9 Krebs R & Burvant M U Nephrotic syndrome in association with varicella *JAMA 222* 325 1972
10 Lopez R & Luhby A L Thrombocytopenic purpura and chickenpox *Clin Pediatr (Phila) 9* 100 1970
11 Minkowitz S Wenk R Friedman E & al Acute glomerulonephritis associated with varicella infection *Am J Med 44* 489 1968
12 Olesen K H Om Haemorrhagiske varceller *Ugeskr Læg 112* 1557 1950
13 Resnick J & Schanberger J E Varicella reactivation in nephrotic syndrome treated with cyclophosphamide and adrenal corticosteroids *J Pediatr 83* 451 1973
14 Urizar R E Michael A Sicca S et al Anaphylactoid purpura II Immunofluorescent and electron microscopic studies of the glomerular lesions *Lab Invest 19* 437 1968
15 Vernier R L Worthen H G Peterson R D & al Anaphylactoid purpura I Pathology of the skin and kidney and frequency of streptococcal infection *Pediatrics 27* 181 1961
16 Yuceoglu A M Berkovich S & Minkowitz S Acute glomerulonephritis as a complication of varicella *JAMA 202* 879 1967

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CASE REPORT

RETROLENTAL FIBROPLASIA OR CONGENITAL ENCEPHALO OPHTHALMIC DYSPLASIA?

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ABSTRACT Svedbergh B (Department of Ophthalmology University of Uppsala Uppsala Sweden) Retrolental fibroplasia or congenital encephalo-ophthalmic dysplasia? Acta Paediatr Scand 64 891 1975.—Congenital encephalo-ophthalmic dysplasia (CEOD) is described in 2 cases The eye signs clinically identical to the cicatricial stage of retrolental fibroplasia (RLF) are combined with brain maldevelopment and occurred without oxygen treatment RLF in a strict sense should be reserved for oxygen induced retinopathy in prematures

KEY WORDS Retrolental fibroplasia congenital encephalo-ophthalmic dysplasia hydrocephalus anencephaly oxygen malformation

Despite restrictions on oxygen treatment quite a few cases of retrolental fibroplasia (RLF) still occur (32 37) Other possible etiological agents must be considered and reports of RLF cases without oxygen treatment are thus of special interest (5 7 9 13 16 17 24 33 36 37) Two other types of deoxygenation have been suggested as the cause of RLF namely intra uterine hypoxia (6 13 17 35 37) and postnatal asphyxia (5 21 38) Other causes than deoxygenation remain obscure Furthermore the differential diagnosis may raise problems both in the cicatricial phase of RLF (29 36 39 41) and in the active phase Thus retinal neovascularization in infants is a non specific secondary reaction occurring not only in RLF but also in a number of pathological conditions such as Coat s disease Norrie s disease falciform retinal detachment intra ocular hemorrhage and others (9 36) It has recently been emphasized in an editorial in the *British Journal of Ophthalmology* (9) that the term RLF should be wholly reserved for oxygen induced retinopathy in prematurity so that all other intraocular conditions in infancy characterized by retinal neovascularization which in all probability are etiologically dissimilar should be set aside until their true nature can be recognized

The purpose of the present report is to revive such a condition namely congenital encephalo ophthalmic dysplasia (CEOD) first described by Krause in 1946 (25) The eye abnormalities were similar to classical RLF whereas he described hydrocephalus microcephaly cerebral agenesia and mental retardation as the usual brain abnormalities However the justification of this separate disease entity has been questioned ever since oxygen was found to be the main cause of RLF in 1952 (26)

In Sweden a survey of 45 RLF patients born in 1960–66 was recently presented (37) Two of these patients also had cerebral abnormalities but did not have oxygen therapy thus suggesting another etiology to the malformations

CASE REPORT

Case 1

J B girl born in 1963 Mother healthy 1 para 2 grav *pregnancy and delivery normal (breech presentation)* Born at 30th gestational week birth weight 1 360 g Oxygen was not administered (except at revival post partum)

Eye abnormalities At 3 months of age the first eye examination showed *microphthalmus* and *extensive* yellow glistening masses in the vitreous body of both eyes diagnosed as RLF cicatricial phase V according to the classification of RLF by Reese et al 1953 (31)

Cerebral abnormalities At 2½ months of age hydrocephalus was suspected and encephalography showed a large cyst infra tentorially in the posterior fossa communicating with the dilated lateral ventricles There was *no gas in the aqueduct and fourth ventricle* Diagnosis Non-communicating hydrocephalus A shunt inoperated between the cyst and the spinal subarachnoid space caused regression of the hydrocephalus

Tests for toxoplasmosis were negative Hearing sense was normal *At 2 years of age cerebrally activated men* strual bleeding occurred Cerebral palsy and mental retardation were later observed

Case 2

B P boy born in 1961 Mother healthy 1 para 2 grav She was exposed to rubella during early pregnancy but without any clinical symptoms Previous exposure to rubella unknown Delivery by sectio due to an X ray diagnosis of intra uterine hydrocephalus Born at 41st gestational week birth weight 4 950 g No oxygen was administered

Eye abnormalities At 4 months of age the first eye examination showed no microphthalmus The lenses were clear but retrolental membranes were observed (RLF cicatricial phase V)

Cerebral abnormalities The hydrocephalus progressed to a circumference of 64 cm at the age of 7 months No further increase was observed No intracranial calcifications were noted on X ray examination

Tests for toxoplasmosis were negative Hearing sense was normal *This boy is severely handicapped and nursed* at an institution for mentally retarded

A third patient referred to earlier (37) is not included as his eye abnormalities could not be classified as any stage of classical RLF Furthermore his twin brother had retinochoroiditis with sero positive toxoplasmosis

DISCUSSION

When Krause first described congenital encephalo ophthalmic dysplasia (CEOD) in 1946 (25) there was still about 6 years before oxygen treatment was found to be the main cause of RLF Krause actually renamed the concept RLF at that time to CEOD emphasizing the occurrence of brain abnormalities (18 41) Thus 13 of his 18 reported patients were premature they were nursed in an incubator and had no objective brain abnormality (i e excluding mental retardation alone) These 13 patients would today probably be diagnosed as RLF since all the 18 patients had an eye disease similar to or identical with contemporary descriptions of RLF Only 5 patients (cases 6 8, 9 10 and 14) had an objective brain abnormality (hydrocephalus or microcephaly) strictly justifying the term CEOD All were born at full term and oxygen treatment was reported for 2 weeks in one case for 2 days in another case and not at all in 3 cases A similar analysis of Krause s patients was carried out by Reese & Blodi (30) They suggested the term retinal dysplasia to be applied to a patient with eye signs identical to RLF and other malformations in any part of the body They presented 8 case reports of which only one had objective brain abnormality (case 4) Retinal dysplasia nowadays generally refers to a non specific lesion resulting from interference with the normal histogenesis of the developing retina (34 36) A microscopic diagnosis of retinal dysplasia should differentiate between RLF and CEOD but might also be observed as a secondary degeneration in eyes with long standing RLF (4 26 40) Other investigators also considered CEOD to be separate from RLF as a disease entity (4 12 39) and even Krause did so later regarding CEOD to be of prenatal and RLF of postnatal origin (14) Anencephaly and hydranencephaly may in some cases be combined with eye signs identical to RLF (1 2 8 15 27) and may thus be considered as CEOD with extreme brain abnormality

It is proposed here that CEOD should in a strict sense be defined as a disease characterized by eye signs identical to RLF and objective brain abnormality (excluding mental retardation alone) and occurring without oxygen treatment This definition agrees (besides to the above mentioned anencephaly cases) with the patients reported by Krause (25 cases 8 10 and 14) Hellstrom (13) Zim

mermann (42) Karlsberg et al (22) and the present two cases In other cases reported be fore 1952 the oxygen data have been incom plete (4 30) The definition is broad in the sense that it includes all types of brain abnormalities and future research may show that CEOD consists of several etiologically dissimilar diseases However the definition of CEOD excludes doubtful cases i e when ox ygen treatment disguises the disease as RLF or when the brain abnormality is not severe enough to be verified objectively By objec tive brain abnormality is here meant condi tions that can be verified by objective methods of examination (inspection X ray electro encephalogram and others) Mental re tardation alone has not been considered as an objective sign of brain abnormality because it is often very difficult to evaluate in the visually handicapped child (10 41) and furthermore much of the mental retardation is possibly caused by a relative sensory deprivation (10) Mental retardation alone is observed in about 1/3 of RLF patients in recent reports (32 37) It has been suggested that oxygen treatment may cause similar qualitative vascular changes in the brain as in the eye However this hypothesis has not been confirmed by human autopsies or animal experiments (3 23 28) although Gyllensten (11) found a quantitative decrease of capillaries in the cortex of mice Interesting in this context are also the experi mental findings in mice that maternal anoxia may cause severe malformations of the brain and the eye in the offspring (19 20)

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REFERENCES

1 Add D J Font R L & Manschot W A Pr l fe t et nopathy in anencephal c babies *Am J Ophthalm l 74* 967 197

And rsen S R Bro-Rasmussen F & Tygstrup I Anence phaly related to ocular development and malformat n *Am J Ophthalmol 64* 559 1967

3 Ashton N Ward B & Serpen G Effects of oxygen on developing retinal vessels with particular reference to the problem of retrolental fibroplasia *Br J Ophthalmol 38* 397 1954

4 Bembridge B A & Jackson C R S Pathological examination of nine cases of retrolental fibroplasia *Br Med J II* 1484 1951

5 Blix H The incidence of retrolental fibroplasia in Gothenburg before and after restricted oxygen administration *Acta Ophthalmol (Kbh) 3?* 735 1954

6 Bruckner H L Retrolental fibroplasia—associated with intrauterine anoxia? *Arch Ophthalmol 80* 504 1968

7 Castren J The significance of prematurity on the eye *Acta Ophthalmol (Kbh)* Suppl 44 53 1955

8 Cogan D C Development and senescence of the human retinal vasculature *Trans Ophthalmol Soc UK 83* 465 1963

9 Editorial Retrolental fibroplasia (RLF) unrelated to oxygen therapy *Br J Ophthalmol 58* 487 1974

10 Freedman D A Congenital and perinatal sensory deprivation Some studies in early development *Am J Psychiatry 127* 11 1971

11 Gyllensten L Influence of oxygen exposure on the postnatal vascularization of the cerebral cortex in mice *Acta Morphol Neerl Scand 2* 289 1959

12 Heath P Pathology of the retinopathy of prematuri ty Retrolental fibroplasia *Am J Ophthalmol 34* 1249 1951

13 Hellstrom B Kan den retrolentala fibroplasin elimineras? *Lakartidningen 55* 1768 1958

14 Hepner W R Krause A R & Nardin H E Retrolental fibroplasia (II Encephalo-ophthalmic dysplasia) *Pediatrics 5* 771 1950

15 Hirose K & Yoshioka H Histopathological study on the anenceph lic eyes Report I Histopathol gical finding of retrolental fibroplasia in the anencephalic eyes *Jap J Ophthalmol 1* 106 1957

16 Huggert A Appearance of the fundus oculi in pre maturely born infants treated with and without oxy gen *Acta Paediatr Scand 43* 327 1954a

17 Huggert A In discussion to Blix (1954) *Acta Ophthalmol (Kbh) 32* 736 1954b

18 Ingalls T H Epidemiology of encephalo-ophthalmic dysplasia *J A M A 138* ?61 1948

19 Ingalls T H Curley F J & Prindle R A Anoxia as a cause of fetal death and congenital defect in the mouse *Am J Dis Child 80* 34 1950

20 Ingalls T H Tedeschi C G & Helpern M M Congenital malformations of the eye induced in mice by maternal anoxia *Am J Ophthalmol 35* 311 195?

21 Kalina R E Hobson W A & Morg n B C Retrolental fibroplasia in a cyanotic infant *Pe diatrics 50* 765 1972

22 Karlsberg R C Green W R & Patz A Congeni tal retrolental fibroplasia *Arch Ophthalmol 89* 1 ? 1973

23 Kennedy C Grave G D & Jehle J W Effect of hypoxia on the cerebral circulation of the newborn puppy *Ped at Res 5* 659 1971

24 Kraushar M F Legal implications of retrolental fibroplasia *Arch Ophthalmol 88* 86 1972
25 Krause A C Congenital encephalo-ophthalmic dysplasia *Arch Ophthalmol 36* 387 1946
26 Mann I *Developmental abnormalities of the eye* Br Med Assoc London 1958 2nd ed p 215
27 Manschot W A Eye findings in hydrancephaly *Ophthalmologica 162* 151 1971
28 Patz A Oxygen studies in retrolental fibroplasia IV Clinical and experimental observations *Am J Ophthalmol 38* 291 1954
29 Patz A Retrolental fibroplasia In *Selected topics on the eye in systemic disease* Grune & Stratton New York and London 1974 p 15
30 Reese A B & Blodi F C Retinal dysplasia *Am J Ophthalmol 33* 23 1950
31 Reese A B King M B & Owens W C A classification of retrolental fibroplasia *Am J Ophthalmol 36* 1333 1953
32 Seedorff T Retrolental fibroplasi som blindheds årsag *Nord Med 79* 269 1968a
33 Seedorff T Retrolental fibroplasia *Acta Ophthalmol (Kbh) 46* 500 1968b
34 Silverstein A M Retinal dysplasia and rosettes induced by experimental intrauterine trauma *Am J Ophthalmol 77* 51 1974
35 Speert H Blodi F C & Reese A B Retrolental fibroplasia a hazard of premature birth *Am J Obstet Gynecol 59* 246 1950
36 Stefani F H & Ehalt H Non-oxygen induced retinitis proliferans and retinal detachment in full term infants *Br J Ophthalmol 58* 490 1974
37 Svedbergh B & Lindstedt E Retrolental fibroplasia in Sweden *Acta Paediatr Scand 62* 458 1973
38 Szewczyk T S Retrolental fibroplasia and related ocular diseases *Am J Ophthalmol 36* 1336 1953
39 Unsworth A C Retrolental fibroplasia or ophthalmic dysplasia of premature infants *Trans Am Opthalmol Soc 47* 738 1949
40 Wadensten L A case of bilateral non attachment of the retina *Acta Ophthalmol (Kbh) 25* 61 1947
41 Zacharias L Retrolental fibroplasia A survey *Am J Ophthalmol 35* 1426 1952
42 Zimmerman L In discussion to A E Maumenee Pathogenesis of congenital glaucoma *Trans Am Ophthalmol Soc 60* 159 1962

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24 Kraushar M F Legal implications of retrolental fibroplasia *Arch Ophthalmol 88* 86 1972
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